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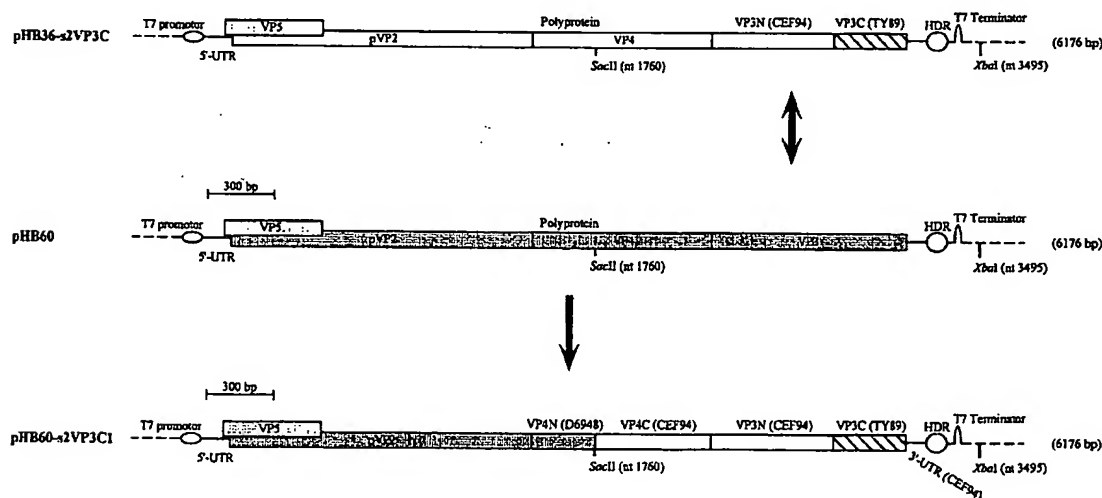
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(54) Mosaic Infectious Bursal Disease Virus vaccines

(57) The invention relates to recombinant Infectious Bursal Disease Virus (IBDV) and vaccines derived thereof. The invention provides infectious recombinant Infectious Bursal Disease Virus (rIBDV) essentially in-

capable of growing in a cell that is not derived from a bursa cell, or an infectious rIBDV having retained at least part of the very virulent characteristics of a very virulent Infectious Bursal Disease Virus (vvIBDV).

Fig. 5g Schematic representation of the construction of plasmid pHB60- α 2VP3C1



Description

[0001] The invention relates to Infectious Bursal Disease Virus (IBDV) vaccines.

Infectious Bursal Disease (IBD), an infectious disease among young chickens, was first recognized in 1957 in Gumboro, Delaware, USA and formally documented by Cosgrove (Cosgrove, 1962; Lasher and Shane, 1994). As a result, the disease is often referred to as Gumboro. Not long after IBD was first reported, it was being recognized in poultry populations throughout the world (Lasher and Shane, 1994). IBD is caused by a virus (IBDV) classified as a Birnavirus (Dobos et al., 1979). Two different IBDV serotypes exist: serotype I and II (Jackwood et al., 1982; McFerran et al., 1980). Isolates belonging to serotype I are highly pathogenic for chickens. Serotype II isolates, which are mainly recovered from turkeys, have never been reported to induce clinical signs in chickens and are regarded as apathogenic (Ismail et al., 1988). Infectious bursal disease or Gumboro is a highly contagious disease for young chickens, and is responsible for severe losses in poultry industries. In birds surviving an acute infection, lymphoid cells in the bursa of Fabricius are destroyed, resulting in B-cell dependent immunodeficiency. This causes increased susceptibility to disease caused by otherwise harmless agents. A central role in the pathogenesis of Gumboro is played by the bursa, which is representing the target organ of the virus.

[0002] IBDV infections were initially recognized by whitish or watery diarrhea, anorexia, depression, trembling, weakness, and death. This clinical IBD was generally seen in birds between three and eight weeks of age. The course of the disease runs approximately 10 day in a flock. Mortality usually ranges from 0-30 percent. Field reports suggest that leghorns are more susceptible to IBDV than broiler type chickens. Subclinical IBD was later recognized and is generally considered a greater problem in commercial poultry than the clinical disease. It is generally seen in birds less than three weeks of age. This early infection results in a B-lymphocyte depletion of the bursa of Fabricius. The bird is immunologically crippled and unable to respond fully to vaccinations or field infections. In susceptible chickens, damage caused by IBDV can be seen within two to three days after exposure to virulent virus. Initially, the bursa swells (3 days post-exposure) with edema and hemorrhages and then begins to show atrophy (7-10 days). IBD virus is especially cytopathic to certain B-lymphocytes. The highest concentration of these specific B-lymphocytes is found in the bursa. Destruction of the B-lymphocytes by IBD field virus may result in an incomplete seeding of these cells in secondary lymphoid tissue. As a result of the depletion of B-lymphocytes, surviving birds are immunocompromised during the remaining of their live time.

[0003] IBDV is found worldwide, and IBDV specific antibodies have even been found in Antarctic penguins (Gardner et al., 1997). The prevalence of clinical IBD is relatively low compared to the prevalence of subclinical IBD. IBDV is very resistant to common disinfectants and has been found in lesser mealworms, mites, and mosquitoes. These facts correlate with field experience of reoccurring IBD problems on a farm, despite clean-up efforts. Infection with IBDV results in a strong antibody response against IBD, which is capable of neutralizing this virus. Most likely as a result of vaccination, antigenic variant isolates of serotype I were isolated in the Delaware area (USA). These isolates have been shown to cause bursa atrophy in as little as three days post-infection without inflammation of the bursa. Despite their change in antigenicity these antigenic variants do not form a distinct serotype. After the occurrence of antigenic variant IBDV isolates in the USA, the poultry industry in European countries was hit by outbreaks of IBD caused by a very virulent serotype I IBDV (vIBDV) (Berg et al., 1991; Chettle et al., 1989; Kouwenhoven and Van den Bos, 1995). These very virulent field isolates were capable of establishing themselves in the face of high levels of maternal antibodies which normally were protective. These vIBDV cause more severe clinical signs during an outbreak and are now found globally (e.g. Europe, Japan, Israel and Asia).

[0004] IBDV belongs to the family of Birna viruses which include Infectious Bursal Disease Virus (IBDV) isolated from chickens, Infectious Pancreatic Necrosis Virus (IPNV) isolated from Fish, Drosophila X Virus (DXV) isolated from fruit fly, and Tellina virus (TV) and Oyster Virus (OV) both isolated from bivalve molluscs (Dobos et al., 1979). Birna viruses have a dsRNA genome which is divided over two genome segments (the A- and B-segment). The A-segment (3.3 kbp) contains two partly overlapping open reading frames (ORFs). The first, smallest ORF encodes the non-structural Viral Protein 5 (VP5, 17 kDa). The second ORF encodes a polyprotein (1012 amino acid, 110 kDa), which is autocatalytically cleaved. The exact position of these cleavage sites is unknown. From SDS-Page analysis of *in vitro* translated IBDV RNA it is known that the polyprotein is rapidly cleaved into three proteins: pVP2 (48 kDa), VP4 (29 kDa) and VP3 (33 kDa). During *in vivo* virus maturation pVP2 is processed into VP2 (38 kDa), probably resulting from site-specific cleavage of the pVP2 by a host cell encoded protease (Kibenge et al., 1997). VP2 and VP3 are the two proteins that constitute the single shell of the virion. The B-segment (2.9 kbp) contains one large ORF, encoding the 91 kDa VP1 protein. This protein contains a consensus RNA dependent RNA polymerase motive (Bruenn, 1991). Furthermore, this protein has been reported to be linked to the 5'-ends of the genomic RNA segments (Viral Protein genome-linked, VPg). The nucleotide sequence of internal parts of a large number of IBDV isolates of classical, antigenic variant or very virulent origin has been determined, and deposited in several databases such as GenBank. Furthermore Mundt and Muller (Mundt and Muller, 1995) have determined the 5'- and 3'-termini of several IBDV isolates (CU-1, CU-1M, P-2 and 23/82), and by combining the internal and terminal sequences, Mundt and Muller established

the complete nucleotide sequence of a serotype I A-segment (3261 bp) and B-segment (2827 bp). This provided the way to generate an infectious (recombinant) copy (rIBDV) of IBDV serotype I, by knowing the complete sequence dsRNA sequence of IBDV genome and by using one of several methods to generate infectious copy virus (see for example Boyer et al, Virology 198:415-426, 1994), Mundt and Vakharia indeed produced infectious rIBDV serotype I from cDNA (Mundt and Vakharia, 1996). Full length cDNA of a serotype I IBDV, preceded by a T7 promoter, was thereby used as a template for T7 RNA polymerase using a method described by Weiland and Dreher (Weiland and Dreher, 1989). The *in vitro* generated mRNA, containing a cap-structure at its 5'-end, was subsequently transfected into eukaryotic cells (VERO cells) using a liposome formulation (Lipofectin, GibcoBRL). The supernatant of the transfected cells contained infectious rIBDV after incubation during 36h in the CO₂ incubator at 37° C (Mundt and Vakharia, 1996; (WO 98/09646)). In addition, Lim et al. introduced two amino acid mutations (D279N and A284T) into the cDNA of vvIBDV isolate HK46 (Lim et al., 1999). These mutations were most probably based on data of Yamaguchi et al. (Yamaguchi et al., 1996), which showed that these specific mutations were found in two independent experiments in which very virulent IBDV isolates lost their very virulent character by adaptation and growth on primary CEF cells. Lim et al obtained a rIBDV isolate which possessed the phenotype of a CEF-culture adapted isolate, i.e. a rIBDV isolate which can be propagated, i.e. is able to infect, multiply and be released for further replication, in vvIBDV non-permissive cells such as CEF cells. Note worthy, Lim et al. were unable to produce an infectious vvIBDV isolate using the unmodified cDNA of the HK46 isolate (Lim et al., 1999). Furthermore, although cDNA of IBDV can be used to produce infectious IBDV, the exact mechanism of replication has not been elucidated yet. Data exist which are in support of a semi-conservative genome replication model for Bimaviridae (Bernard, 1980; Mertens et al., 1982).

[0005] Now and then IBDV variants are detected in the field or are created in cell-culture in the laboratory (Muller, 1987) that are genetic re-assortments of serotype I and II strains of IBDV, in that they contain one genomic segment derived from the one serotype, and another segment derived from the other serotype. Such segment reassorted (srIBDV) strains (also called chimeric IBDV) not only occur in nature, but have recently been generated from cDNA as a well, by Vakharia and Mundt (WO 98/09646). Vaccination using attenuated field isolates worked sufficiently well until antigenic derivatives were found in the Delaware region of the USA starting in 1985 (isolates Del A, D, G and E) (Snyder, 1990). These field isolates were missing an important virus neutralizing epitope. The change of this epitope is characterized by the lack of binding of the virus neutralizing monoclonal antibody (Mab) B69 (Snyder et al., 1988a). The antibodies induced by vaccination with classical IBDV vaccines appeared to be less protective against these antigenic IBDV variants. Inactivated vaccines based upon antigenic IBDV variants were subsequently produced and were found to protect effectively against these antigenic variants of IBDV. After the Delaware variant a second antigenic variant IBDV was isolated. This variant was recovered from the Delmarva region (USA) and was referred to as the GLS variant. The GLS variant is characterized by the absence of epitopes for both the virus neutralizing Mab B69 and R63 (Snyder et al., 1988b). After identifying these antigenic variants, a large survey was performed within the USA by using a panel of nine Mabs against IBDV. This survey yielded an additional antigenic variant: the DS326 variant. This antigenic variant is characterized by the absence of epitopes for Mab 179 and BK44, in addition to those for Mabs B69 and R63 (Snyder, 1990). No further reports of antigenic variants have been published in the USA or in other parts of the world. Whether this is due to non-existence of new variant IBDV isolates or whether new antigenic variants just have not been detected due to the lack of extensive surveys or the lack of discriminating monoclonal antibodies is unclear.

[0006] The nucleotide sequence of the polyprotein encoding part of the A-segment of the Del, the GLS and the DS326 antigenic variant IBDV isolates has been determined (Vakharia et al., 1994). Most of the amino acid changes were found in a specific region of the VP2 protein, the so-called hypervariable region. Furthermore it was found that the epitopes which are capable of inducing neutralizing antibodies are conformation dependent and are clustered in the hypervariable region. This region consists of a domain with a high hydrophobicity index (amino acid 224 to 314 of pVP2, corresponding with amino acid 224 to 314 of the polyprotein) which is flanked by two small hydrophilic regions, each spanning about 14 amino acids (Vakharia et al., 1994; Heine et al., 1991). Amino acid substitution both within the hydrophobic region and within the hydrophilic regions might be involved in the antigenic variant character of these isolates.

[0007] After the problems caused by the antigenic variant IBDV isolates in the USA, the poultry industry in Europe was affected by very virulent IBDV (vvIBDV) isolates (Berg et al., 1991; Chettle et al., 1989). The vvIBDV isolates cause more severe clinical signs during an outbreak and are able to break through levels of antibodies which are protective against classical IBDV isolates. The molecular determinants which distinguish vvIBDV from classical IBDV isolates are not exactly known. It is known however, that the pathogenicity of cell culture adapted very virulent IBDV isolates is severely reduced, compared with the non-adapted parental isolates (Yamaguchi et al., 1996). The correlation between CEF-adaptation and loss of the very virulent phenotype is likely to be due to the change in target cell tropism of the adapted virus. This change in cell tropism may be due to the loss of bursa cell receptor binding capability of the cell culture adapted very virulent IBDV isolate. Another possibility is that the cell culture adapted very virulent IBDV isolate is able to infect non-bursa cells, resulting in large reduction of IBDV load in the primary target cells (bursa cells). From the published results (Yamaguchi et al., 1996), it is clear that a recombinant IBDV (rIBDV) which is based upon

the cDNA of a cell culture adapted very virulent isolate will never yield a vaccine which meets the demands of being able to break through high levels of maternal antibodies and induce a high enough immune response.

[0008] No specific antibodies, that exclusively recognize the vvIBDV isolates have been described yet (Eterradossi et al., 1997)). The lack of discriminating antibodies makes direct diagnosis difficult. Most attention has been given to sequence comparison between the hypervariable region of VP2 of classical isolates and of very virulent isolates. Sequence analysis of the vvIBDV isolate UK661 showed that only three unique (i.e. not found in non-vvIBDV isolates) amino acid substitution are present within the hypervariable region of the VP2 protein. One amino acid substitution is present within the remaining part of the pVP2 protein, while 5 unique amino acid mutations are present within the VP4 encoding part of the polyprotein and 6 in the VP3 encoding part. (Brown and Skinner, 1996). The smaller ORF of the UK661 isolate A-segment, encoding the VP5 protein, contains 2 unique amino acid substitutions. Additionally 16 unique amino acid substitutions were found in the VP1 protein encoded by the B-segment of this vvIBDV isolate. The virulent phenotype of the vvIBDV might be influenced by each of the found amino acid substitutions, and even (silent) nucleotide substitutions within the coding or non-coding parts of either the A- or B-segment may contribute to the altered phenotype of the vvIBDV isolates in comparison with the classical or antigenic variant isolates. Serial passage on embryonated eggs of a vvIBDV isolate (OKYM) resulted in the appearance of a derivative isolate (OKYMT) which is able to grow on Chicken Embryo Fibroblast (CEF) cells and has lost its virulence. This adaptation was reported to be the result of 7 nucleotide substitutions in the polyprotein encoding part of the genome. Whether additional nucleotide substitutions (or deletions) were present in remaining parts of the A- or B-segment (e.g. untranslated regions, VP1 encoding region, and VP5 encoding region) was not determined (Yamaguchi et al., 1996). The reported nucleotide substitutions result in 5 amino acid substitutions. Three of these amino acid substitutions were located in the hydrophobic part of the hypervariable region (I256T, D279N, A284T) of VP2, one in the hydrophilic part located downstream of the hypervariable region (S315F) of VP2, and one in VP3 (A805T) (Yamaguchi et al., 1996). In an independent experiment, Yamaguchi et al. found that the adaptation of vvIBDV isolate TKSM into TKSMT resulted also in the A284T and D279N substitutions. The A284T substitution correlated in their analysis completely with adaptation onto CEF cells and loss of virulence. The D279N substitution was also present in both CEF-adapted vvIBDV isolates (OKYMT and TKSMT) and is potentially also important for growth on CEF cells and loss of virulence. The non-CEF adapted, classical IBDV isolate GBF-1 has on the other hand an asparagine at position 279, in combination with alanine at position 284 and cannot grow on CEF cells, so the single substitution D279N does not account loss of virulence and growth on CEF cells. The amino acid changes in the VP2 apparently allow the modified IBDV to propagate on cells which do not have a receptor for wild type IBDV. Cells possessing a wild-type IBDV receptor such as bursa cells are susceptible for classical and vvIBDV isolates. Recently it was shown that amino acid substitution, A284T in combination with D279N is indeed enough to turn a non-CEF-adapted very virulent IBDV isolate into a CEF-adapted isolate. Lim et al. introduced these two amino acid substitutions into the A-segment cDNA of vvIBDV isolate HK46 (Lim et al., 1999). After transfection of this cDNA, Lim et al. obtained a rIBDV isolate which possessed the phenotype of a CEF-culture adapted isolate, i.e. a rIBDV isolate which is able to infect and multiply in CEF cells. The virulence of this rIBDV isolate was not assessed in chickens. Note worthy, Lim et al. were unable to produce a recombinant infectious vvIBDV isolate using the unmodified cDNA of the HK46 isolate (Lim et al., 1999).

[0009] The goal of vaccination against IBD is prevention of subclinical and clinical IBD and the economic aspects of each. Effective vaccination for IBD can be divided into the following categories:

[0010] Protection of the developing bursa in broilers, breeders and layers.

[0011] Prevention of clinical disease in broilers, breeders and layers.

[0012] Priming and boosting of breeders.

[0013] To minimize the immunosuppressive effects of IBDV, the young chick must be protected. Protection of the very young can be achieved through high enough levels of maternal antibodies passed from the breeder hen to her progeny. Vaccination of the very young chick itself may not be successful since onset of protection after vaccination is between three and five days. When a bird, lacking maternal antibodies against IBDV, is exposed to a pathogenic IBDV field strain, damage will occur within 24-48 hours.

[0014] Generally the early vaccinations of the breeders serve as priming. In most situations, this single vaccination is not considered to be adequate. Boosting is the term commonly associated with the administration of a final IBDV vaccination prior to the onset of lay. This is done to increase the circulating antibody in the hen and hence the maternal antibodies in the progeny. Both inactivated (oil emulsion vaccine) and live vaccines (IBDV) have been used for this purpose. The use of a live vaccine in an older bird will result in an increase of antibodies; however, large variations in antibody titers are often seen. These variations result in progeny becoming susceptible to field challenge from as early as a few days after hatching to 21 days after hatching. The use of inactivated IBDV vaccines gives a higher antibody titer as well as a decrease of variation between antibody titers of birds belonging to the same flock. The levels of maternal antibodies necessary to neutralize IBDV vary with the invasiveness and pathogenicity of the field strain. In practical terms, if a very virulent IBDV isolate is present, higher maternal antibody levels are desired (see Table 1 for an overview of virulence of field isolates and strength of vaccines). Yet, for effective vaccination, avoiding interference

with maternal antibodies is needed to induce a good immune response. Clinical IBD is typically seen between three and six weeks of age. The immune response of the chick must be stimulated as the passive protection is declining. The timing of the active vaccination may be estimated by the breeder or chick titer and the half-life of antibodies of approximately 3.5 days (De Wit and Van Loon, 1998; Kouwenhoven and Van den Bos, 1995). The levels of maternal antibodies tend to vary within a population. This variation might be a result of variation in the antibodies levels of the breeder hen. Also the mixing of progeny from several breeder flocks (e.g. combination of breeders of different age; breeders vaccinated with live vaccine and those with oil emulsion vaccine) results in variation of IBDV antibodies between chick belonging to the same flock. If the coefficient of variation (CV) in mean maternal antibody titers is too wide, it may be recommended to vaccinate twice (with a 10-day interval) or to vaccinate early with a hot vaccine (in the presence of a high antigenic pressure).

[0015] The average titer of antibody against IBDV in a flock will decline in time (Fig. 1). As a result of the decrease in average antibody titers, an immunity gap will occur. The best results are obtained if the immunity gap is as short as possible and is as early as possible, with a minimum of 2 weeks after hatching. There should be at least sufficient immunity after active vaccination at the age of 4 weeks, since many handlings occur in the houses from that time point with risks of introducing field virus. Therefore farmers like to vaccinate at 2 weeks or even before. Intermediate vaccines are often unable to break through the average IBDV antibody titer of the broiler at two weeks after hatching (Fig. 1). If there is a high variation in mean maternal antibody titers, some chicks will be effectively vaccinated with intermediate vaccines, others not. To circumvent those problems, hot vaccines are being used. A drawback of usage of hot vaccines is that the bursa of chickens with low to moderate maternal antibody titers will be (partly) damaged.

[0016] There is a wide variety of IBDV vaccines available. Important aspects in vaccination strategies are the ability of the virus to replicate in the face of maternal antibody (invasiveness of the vaccine) and the spectrum of antigenic content (including antigenic variants). The ability of a vaccine virus to replicate in the face of maternal antibodies allows live vaccines to be categorized into three main groups: mild, intermediate, and intermediate plus or hot vaccines (see Table 1). The initial vaccines for IBD were derived from classical IBDV isolates. These vaccines were moderately pathogenic IBDV strains with low passage numbers in embryonated eggs. These were often used in breeder programs to induce high levels of circulating antibodies. However, when given to a young bird with moderate or low levels of maternal antibodies, these vaccines could cause extensive bursal atrophy resulting in immunosuppression. Mild vaccines were subsequently developed to be used in these young birds. The attenuation of classical IBDV was done in tissue culture systems. Traditionally, attenuated strains for vaccines are generated by adapting IBDV strains to chicken embryoblast (CEF) cells or other appropriate cells or cell lines through serial passages. These vaccines are not immunosuppressive even when used in birds having no maternal antibodies. However, moderate and high levels of antibodies easily neutralize them. As breeder programs developed (including the use of adjuvant, inactivated vaccines), higher levels of maternal antibodies were generated in progeny. This reduces the effectiveness of these mild vaccines.

[0017] Intermediate strength vaccines were to overcome the inadequacies of the mild vaccines. Some of the intermediate vaccines were developed by cloning a field isolate on chicken cell cultures. Intermediate strength vaccines are capable of establishing immunity in birds with moderate levels of maternal antibodies. These vaccines will cause some bursal atrophy in birds without maternal antibodies, but are considered not immunosuppressive.

[0018] Hot (strong) or intermediate plus vaccines were developed after the first outbreaks with vvIBDV. These vvIBDV isolates could break through higher levels of maternal immunity than the vaccines that were on the market at that time. Vaccination with intermediate vaccines came always too late in situations with high infection pressure with vvIBDV. Hot vaccines consist of vvIBDV strains with low to moderate passage in embryonated eggs or bursa derived IBDV of chickens infected with vvIBDV isolates. Adapting vvIBDV on cells traditionally used for the generation of vaccines in general fails, since either these cells are non-permissive for vvIBDV, or, when adapted to said cells, the vvIBDV in question had lost its very virulent character, making it useless for hot or intermediate plus vaccine. Hot or intermediate plus vaccines are desirably able to circumvent maternal immunity at an earlier age than intermediate vaccines but spread more within a flock. If intermediate plus and hot vaccines are used in chickens with moderate to high levels of maternal antibodies, there is no negative side effect on the bursa (Kouwenhoven and Van den Bos, 1995). If these vaccines are used in chickens with low to moderate levels of maternal immunity, this causes depletion of lymphoid cells in the bursa and a severe depletion of peripheral blood-B cells is found (Ducatelle et al., 1995). Although a recovery of bursal function has been observed, these vaccines should be used with precautions.

[0019] Live vaccines must be given in a way in which the virus will preferably reach the bursa where it will quickly multiply and induce an immune response. Possible routes for application of live vaccines include drinking water, spray, subcutaneous and *in ovo*. Inactivated IBD vaccines are used in broiler breeders. They differ in some of the same ways as live vaccines. Their efficacy depends upon the spectrum of antigens they contain. Injectable oil-emulsion products may be given subcutaneously or intramuscularly.

[0020] A continuous monitoring of the field situation using an integrated quality control scheme including serology, can be a valuable tool for continuously adapting preventive vaccination programs to changing epidemiological conditions. Also a continuous follow-up of the epidemiological situation will allow to anticipate the development of major

epidemics (Ducatelle et al., 1995). However, the ability of diagnostic laboratories to monitor IBD with meaningful definitive data is difficult. Serology is important but can be confusing when all birds monitored from commercial broiler flocks have high levels of the same spectrum of circulating antibodies. Field evaluations of broilers to monitor the status of IBD are highly subjective: it is difficult to discriminate antibody titers obtained after vaccination from those induced by IBDV field infections. If it were possible to discriminate between IBDV antibody response to field virus and IBDV vaccination it is possible to have 'early warning' systems and to start IBDV eradication programs if desired. Only when there is a known difference between the antibody response to the used IBDV vaccine and IBDV field isolates, defined conclusion about whether (sub)clinical signs of IBDV are the result of live IBDV vaccination or of IBDV field isolates can be made.

[0021] The invention provides infectious recombinant Infectious Bursal Disease Virus (rIBDV) essentially incapable of growing in a cell that is not derived from a bursa cell or another cell comprising a wild-type IBDV receptor (a non-bursa-cell). A bursa is lymphoid organ, mostly comprising cells that are related to the immune system. In particular, it comprises lymphocytes or lymphocyte precursor cells of sometimes the T-cell- but mainly the B-cell-type, and cells derived thereof, in close relation with monocytes or monocyte derived cells such as macrophages, and also with follicular dendritic cells and antigen presenting cells. In particular, the invention provides rIBDV that is essentially incapable of growing in a cell not listed among above bursa cells or cells derived thereof, such as dendritic cells, monocytes, lymphocytes or cells derived thereof. Herewith the invention provides an rIBDV having retained an important characteristic, in that, an comparison with commonly attenuated IBDV strains, it can not or only little grow in non-bursa cells, such as the well known CEF, QM5 or VERO cells, or other cells that are commonly used for propagating attenuated strains of IBDV. In particular, the invention provides an rIBDV essentially incapable of growing in a non-B-cell derived cell. Essentially incapable of growing herein means that the isolate in question is not or only little capable to infect, multiply or be released for further replication. No such rIBDV isolates exist prior to this invention, all previous rIBDV isolates known grow in non-bursa-cell derived cells such as CEF cells (W098/09646; Lim et al., 1999), thereby for example having lost those very virulent characteristics essential for maintaining in a vaccine strain designed to face above identified problems.

[0022] In a preferred embodiment the invention provides in infectious rIBDV having retained at least part of the very virulent characteristics of a very virulent Infectious Bursal Disease Virus (vvIBDV) needed to provide protection against vvIBDV. In particular, vvIBDV is provided that is essentially incapable of growing in a non-bursa-cell derived cell. In particular, as for example demonstrated in the detailed description, the invention provides an rIBDV essentially incapable of growing in a CEF cell, a VERO cell or a QM5 cell, except of course in those CEF, VERO, QM5, or related cells having been provided with the necessary means (such as transgenic receptor or replication system derived from a bursa-cell) needed for replication of classical or very virulent IBDV.

[0023] Furthermore, the invention provides an rIBDV wherein the amino acid sequence of protein VP2 comprises no asparagine at amino acid position 279, but for example comprises an amino acid particular for a strain with a very virulent character, such as with aspartic acid at amino acid position 279. Such rIBDV strains as provided by the invention have retained at least part of the very virulent characteristics of vvIBDV, as well as an rIBDV according to the invention wherein the amino acid sequence of protein VP2 comprises no threonine at amino acid position 284, but for example comprises an amino acid particular for a strain with a very virulent character, such as with alanine at amino acid position 284.

[0024] In a preferred embodiment, the invention provides an rIBDV according to the invention wherein the amino acid sequence of protein VP2 at least comprises a stretch of amino acids from about position 279 to 289, preferably from about position 229 to 314, most preferably from about position 214 to 328 as found in a vvIBDV isolate such as shown in Table 8.

[0025] The invention furthermore provides a method for obtaining an infectious recombinant copy Infectious Bursal Disease Virus (rIBDV) essentially incapable of growing in a non-bursa-cell derived cell or having at least part of the very virulent characteristics of a very virulent Infectious Bursal Disease Virus (vvIBDV) comprising transfecting at least one first cell with a nucleic acid such as a cDNA or RNA comprising an IBDV genome at least partly derived from a vvIBDV, incubating said first cell in a culture medium, recovering rIBDV from said transfected first cell or said culture medium, propagating said recovered rIBDV in at least one second cell which is permissive for said vvIBDV. A vaccine derived of the recombinant virus as described is also part of this invention. Also a vaccine comprising a chemically or physically inactivated recombinant virus or parts thereof is part of this invention.

[0026] Also the attenuated derivatives of initially produced recombinant very virulent IBDV are part of this invention. Such a virus can be attenuated by known methods including serial passage, removing specific nucleic acid sequences, or by site directed mutagenesis. Physiologically acceptable carriers for vaccines of poultry are known in the art and need not be further described herein. Other additives, such as adjuvants and stabilizers, among others, may also be contained in the vaccine in amounts known in the art. Preferably, adjuvants such as aluminum hydroxide, aluminum phosphate, plant and animal oils, and the like, are administered with the vaccine in amounts sufficient to enhance the immune response to the IBDV. The vaccine of the present invention may also contain various stabilizers. Any suitable

stabilizer can be used including carbohydrates such as sorbitol, mannitol, starch, sucrose, dextrin, or glucose; proteins such as albumin or casein; and buffers such as alkaline metal phosphate and the like. A stabilizer is particularly advantageous when a dry vaccine preparation is prepared by lyophilization. The vaccine can be administered by any suitable known method of inoculating poultry including nasally, ophthalmically, by injection, in drinking water, in the feed, by exposure, and the like. Preferably, the vaccine is administered by mass administration techniques such as *in ovo* vaccination, by placing the vaccine in drinking water or by spraying the animals' environment. When administered by injection, the vaccines are preferably administered parenterally. The vaccine of the present invention is administered to poultry to prevent IBD anytime before or after hatching. Poultry is defined to include but not be limited to chickens, roosters, hens, broilers, roasters, breeders, layers, turkeys and ducks. Examples of pharmaceutically acceptable carriers are diluents and inert pharmaceutical carriers known in the art. Preferably, the carrier or diluent is one compatible with the administration of the vaccine by mass administration techniques. However, the carrier or diluent may also be compatible with other administration methods such as injection, eye drops, nose drops, and the like.

[0027] As explained above, there is need for an IBDV vaccine that can protect against field infections with IBDV, and preferably against very virulent IBDV (vvIBDV). It is clear that vaccines derived from attenuated classical strains and not from very virulent strains will not be able to sufficiently protect. However, as explained above, simply by adapting and cultivating a vvIBDV strain on a cell or cell-line, such as VERO, CEF or QM5, as one skilled in the art would first do in order to obtain a vaccine strain from a vvIBDV strain, reduces its virulent phenotype such that no sufficient protection is to be expected. Therefore, a vaccine strain is needed that has at least partly maintained the very virulent or hot character, in order to provide sufficient protection, however, paradoxically, such a desirable vaccine strain would most likely not be able to be sufficiently or substantially be propagated on appropriate cells, such as non-bursa-cell derived VERO, CEF or QM5, deemed needed to obtain said vaccine. In a preferred embodiment, the invention provides a method wherein said first cell is a non-bursa-cell derived cell non-permissive for said vvIBDV, preferably wherein said first cell has additionally been provided with a helpervirus or a viral protein (herein T7-polymerase is used) derived thereof. With the help of such a cell comprising a properly selected helpervirus, e.g. expressing distinct IBDV or Birna virus viral proteins, or of a cell expressing said IBDV or Birna virus viral proteins, (also called a complementary cell) also now defective or deficient rIBDV can be made.

[0028] The invention therewith also provides a method to generate infectious Infectious Bursal Disease Virus, by combining cDNA sequences derived from very virulent IBDV (vvIBDV) isolates with cDNA sequences derived from either serotype I classical attenuated IBDV isolates, serotype I antigenic variants of IBDV, or serotype II IBDV isolates, wherein said infectious copy recombinant Infectious Bursal Disease Virus having retained at least part of the very virulent characteristics of a very virulent Infectious Bursal Disease Virus has at least retained the incapacity to substantially be propagated on a vvIBDV non-permissive cell such as a QM5 or CEF cell.

[0029] Preferably, a method as provided by the invention provides a vaccine comprising an IBDV genome wherein parts of segments A and/or B derived from a vvIBDV are used combined with parts of segments A and/or B derived from an attenuated IBDV, such as attenuated serotype I or I IBDV. Such a rIBDV is herein also called a mosaic IBDV (mIBDV). Herein we show that (mosaic) vvIBDV can be generated from cDNA by transfection of non-susceptible cells followed by amplification of the cDNA-derived rIBDV on susceptible cells. The method provided herein provides a method to generate wIBDV from the cloned, full length cDNA of a wIBDV isolate (see Table 5 and 6). After transfection of QM5 cells with cDNA of wIBDV it is essential that propagation of the generated wIBDV virus takes place on cells which are permissive for wIBDV. These permissive cells can for example be found among Bursa-cell derived cells such as primary bursa cells, in chicken in embryo cells, chicken embryo's, or young chickens. Using the method described herein we have for example produced recombinant D6948 (rD6948) using the cDNA derived from the very virulent D6948 IBDV isolate. This rD6948 isolate has the same virulence as the parental D6948 isolate (Table 6).

[0030] Preferably, the invention provides a method wherein a permissive second cell is a primary bursa cell, thereby allowing initial propagation of the desired vaccine virus. As explained above, there is a need for a vaccine capable of breaking through maternal immunity of young chickens at an early stage. A desired vaccine should preferably be able to induce a high level of protection in vaccinated young chickens, and should therefore be as immunogenic as very virulent viruses or be almost as immunogenic.

[0031] The invention furthermore provides a method to engineer recombinant mosaic IBDV (mIBDV) vaccine which has one or more of the desired phenotypes, i.e. i) being able to break through high levels of maternal antibodies in young chickens and being highly immunogenic, ii) having a reduced pathogenicity compared to wild type very virulent IBDV isolates. In one embodiment, the invention provides an infectious mosaic IBDV (mIBDV) comprising a rIBDV wherein at least one genome segment comprises nucleic acid derived from at least two different Birna virus isolates, when preferred wherein at least one of said isolates is a wIBDV characterised by its incapacity to substantially be propagated on a vvIBDV non-permissive cell such as a VERO, QM5 or CEF cell and/or characterised by its capacity to substantially be propagated on a vvIBDV permissive cell such as a primary bursa cell. For example, the invention provides mIBDV which consists partly of the genome derived from a classical attenuated isolate (such as CEF94) and partly derived from the genome of a vvIBDV isolate (such as D6948). A recombinant mosaic IBDV (mIBDV), made on

the basis of infectious cDNA derived from a very virulent IBDV isolate (D6948), and combined with defined parts of cDNA derived from a cell culture adapted, serotype I, classical IBDV isolate (CEF94) results in a mIBDV isolate which has a reduced pathogenicity compared to wild-type vvIBDV isolates.

[0032] Furthermore specific nucleotide substitutions which either do or do not lead to amino acid mutations, or deletion of specific parts of the IBDV genome again leads to an altered phenotype of the generated mIBDV. For example, the replacement of the pVP2 coding region of CEF-94 cDNA with the corresponding region of cDNA of D6948 yielded plasmid pHB36-vvVP2. This plasmid was subsequently transfected into FPT7 (Britton et al., 1996) infected QM5 cells in combination with pHB-34Z. Supernatant of these transfected QM5 cells was subsequently transferred to fresh QM5 cells. None of these QM5 cells reacted positively in an IPMA using specific antibodies for the VP3 protein of IBDV. On the other hand, primary bursa cells, after being overlaid with supernatant of the transfected cells, reacted positively in the same IPMA. The functional feature of being able to enter permissive cells such as QM5 cells is apparently located in the pVP2 coding region of the A-segment. This invention provides a method to generate recombinant vvIBDV (such as rD6948) having a pVP2 sequence exactly as found in a wild-type vvIBDV (here D6948). All very virulent isolates of which the pVP2 sequences has been described thus far have an alanine at position 284 and cannot or only little be propagated on CEF cells (see Table 7 and 8). On the other hand, when a threonine is present at position 284, propagation on CEF cells is possible, but this is associated with the lack of a very virulent phenotype (see Table 7 and 8). Herein we describe a method to generate infectious recombinant IBDV (rIBDV) having the nucleotide sequence of a wild-type very virulent IBDV isolate, including the alanine codon for amino acid 284, and being unable to be propagated on CEF cells. Furthermore, in our rD6948 isolate we have an aspartic acid present at position 279 instead of a asparagine commonly found for avirulent IBDV isolates which can be propagated on CEF cells (Table 7 and 8). The rD6948 is truly a very virulent rIBDV, as it is unable to grow on CEF cells (Table 5), and induces similar clinical signs and mortality as wild-type very virulent D6948 isolate (Table 6). Although mIBDV isolate (mCEF94-vvVP2) did, in contrast to the D6948, rD6948 and srIBDV-DACB isolates (also having a functional VP2 protein derived from vvIBDV, see Table 6), not cause any mortality in a 9-days course or body weight loss, it caused the same reduction in bursa weight after 9 days post-infection as the wild-type very virulent D6948 isolate.

[0033] In yet another embodiment, the invention provided a mosaic IBDV according to the invention wherein at least one of said isolates is a serotype II IBDV. Such a mIBDV, preferably lacking at least one immunodominant epitope specific for a serotype I IBDV as well as a (r)D6948 derived vaccine virus such as mD6948-s2VP3C1, also having a functional VP2 protein derived from vvIBDV, allowing vaccination with a marker vaccine. Vaccination with a IBDV marker vaccine and subsequent testing with a corresponding diagnostic test enables the discrimination between antibodies induced by the vaccine and by infection with IBDV field isolates. This mIBDV can be differentiated from all other known wild-type IBDV isolates, either belonging to serotype I or serotype II, for example by using a specific set of monoclonal antibodies. The generation of mIBDV from serotype I and II cDNA provides such a mIBDV marker vaccine that induces a serological response in chickens that can be differentiated from the serological response induced by IBDV field strains. The marker vaccine provided by the invention, lacking at least one immunodominant epitope, preferably a serotype I epitope, enables the discrimination between vaccinated and infected animals by means of a diagnostic serologic test. Such a mIBDV marker vaccine is preferably based upon vvIBDV and contains specific sequences originating from classical serotype I or serotype II IBDV. Such a mIBDV marker vaccine has one or more of the following characteristics: i) It induces a protective immune response against vvIBDV field viruses despite high levels of maternal antibodies. ii) It has a reduced pathogenicity compared to vaccines based upon wild-type vvIBDV. iii) It for example misses at least one serotype I specific antigen which enables the serological discrimination of the mIBDV marker vaccine from all serotype I IBDV isolates.

[0034] Also the invention provides a method to produce or generate tailor made vaccines against specific antigenic variants of IBDV by incorporating the specific amino acid changes in a mIBDV vaccine virus. Depending on the composition, these mosaic IBD viruses (mIBDV) possess different phenotypes and different antigenic properties. A specific mutation in one of the viral proteins can have a profound effect on IBDV viability. We found that this is true in case of a single nucleotide substitution, leading to a single amino acid mutation in VP4 (V582A). No rIBDV could be rescued from cDNA when this particular nucleotide substitution was present. Not only mutations within the VP4 encoding region itself, but also mutations or deletions in the region of the cleavage sites (pVP2-VP4 and VP4-VP3) may have a negative effect on replication of rIBDV. Mutations in the other viral proteins, or even deletion of an entire viral protein (i.e. VP5) influences the replication and or virulence as well. Two groups have constructed an VP5 minus rIBDV isolate, by introducing mutations in the cDNA of an CEF-adapted D78 IBDV isolate (Mundt et al., 1997; Yao et al., 1998). Apparently the VP5 protein, which is a non-structural protein, is also a non-essential protein. Yao et al. reported that inactivation of the ORF for VP5 (replacement of the startcodon by a stopcodon) yielded infectious rIBDV (rD78NS which grows to slightly lower titers (*in vitro*) than rD78, while Mundt et al. reported that inactivation of the ORF for VP5 (replacement of the startcodon by a arginine codon) yielded a rIBDV (IBDV/VP5-) which is able to grow to the same titers (*in vitro*) as the parental isolate. Furthermore Yao et al. reported that rD78NS has a decreased cytotoxic and apoptotic effects in cell culture (*in vitro*) and has a delay in replication compared to the parental isolate (*in vivo*), and

failed to induce any pathological lesions or clinical signs of disease in infected chickens.

[0035] Mutations or deletions in the mIBDV cDNA yields a mIBDV with a desired phenotype, i.e. mIBDV which is based on a very virulent isolate but which has a reduced ability to replicate and hence an reduced pathogenicity. The introduction of cDNA sequences from a serotype II, cell culture adapted, IBDV isolate (TY89) into the mosaic virus gives us yet another opportunity to generate marker mIBDV vaccine which can be discriminated from wild-type serotype I IBDV, for example by using specific monoclonal antibodies. Such mIBDV can be used to induce an antibody spectrum, which differs from the spectrum induced by IBDV field isolates. This enables the development of a serologic test to determine whether IBDV antibodies are the result of live mIBDV vaccination or of infection with IBDV field isolates. For example, the mCEF94-s2VP3C virus is recognized by serotype II specific VP3 antibody (Mab T75) while it is also recognized by a serotype I specific VP2 antibody (Mab 1.4). This particular rIBDV is, on the other hand, not recognized by a serotype I specific VP3 antibody (Mab B10). No apparent difference is present between the replication of mCEF94-s2VP3C and rCEF94, indicating that the exchange of the VP3C-terminal part does not lead to major changes in replication ability in QM5 cells. When, on the other hand, the complete VP3 encoding region was exchanged we observed a severe reduction in replication ability of the resulting virus (mCEF94-s2VP3). On the other hand, mCEF94-s2VP3N was not reacting with Mab C3 (VP3, serotype I) while it is fully reacting with Mab B10 (VP3, serotype I) and only partially with Mab T75 (VP3, serotype II). Replication of this mosaic IBDV on CEF cells is reduced compared to rCEF94. From the generated mIBDV, based on cDNA derived from serotype I (CEF94) and serotype II (TY89), it is clear that a serological marker based on VP3 has been identified. The replacement of the cDNA of (part of) VP3 of serotype I for the corresponding part of serotype II, leads to an unique combination of IBDV antigens within one mIBDV isolate. An mIBDV isolate based on this combination of antigens can be used as an IBDV-marker vaccine.

[0036] The introduction of the VP3 C-terminal part of TY89 (Serotype II) into the cDNA of D6948 yielded a mosaic IBDV (mD6948-s2VP3C1) which has a reduced virulence (no mortality, no body weight loss) compared to D6948 or rD6948 (Table 6). This mIBDV, or a comparable isolate which is more or less virulent, is also advantageously used as an IBDV marker vaccine to prevent infections with very virulent IBDV field isolates.

[0037] Furthermore, the invention provides using site-specific mutagenesis techniques to introduce any desired nucleotide mutation within the entire genome of mIBDV. Using this technique allows adapting mIBDV vaccines to future antigenic variations by including any mutation that has been found in antigenic variant IBDV field isolates. Furthermore, it is provided by the invention to exchange part of the genomic sequence of IBDV with the corresponding part of a Birna virus belonging to another genus (e.g. DXV, IPNV, OV, TV). Herewith, the invention provides new mosaic Birna (mBirna) viruses which have new characteristics resulting in new recombinant vaccines for IBDV or other Birna viruses. Also the use of cDNA of other Birna viruses (e.g. DXV, IPNV, OV or TV) leads to new IBDV vaccines. In this approach, one or more of the IBDV immunodominant or neutralizing epitopes are exchanged with the corresponding parts of the protein of another Birna virus.

[0038] Of course, the invention also provides a method for producing an rIBDV according to the invention, said vector comprising heterologous nucleic acid sequences derived from another virus, or (micro)organism, whereby r- or mIBDV serves as a vector. For example a method is provided to generate an infectious copy IBDV which expresses one or more antigens from other pathogens and which can be used to vaccinate against multiple diseases. Such an infectious copy IBDV for example comprises a heterologous cDNA encoding a heterologous protein obtained from a pathogen, for example poultry pathogens. Also a method is provided to generate a conditional lethal IBDV deletion mutant which can be used as self-restricted non-transmissible (carrier) vaccine. Such an IBDV deletion mutant is unable to express one of the IBDV proteins, and is phenotypically complemented by supplying the missing protein by other means.

[0039] The invention is further explained in the detailed description without limiting the invention thereto.

Detailed description

Material and Methods

Viruses and cells

[0040] The IBDV isolate CEF94 is a derivative of PV1 (Petek et al., 1973). After receiving the PV1 isolate in our laboratory in 1973, we have further adapted this isolate by repeated passage (> 25 times) on either primary Chicken Embryo Fibroblast (CEF) cells or Bursa cells. The very virulent IBDV isolate D6948 was originally isolated in 1989 by the Poultry Health Service (Doorn, the Netherlands). It was purified by 5 passages in embryonated eggs and one subsequent passage in SPF leghorn chickens. IBDV Serotype II isolate TY89 (McFerran et al., 1980) was maintained in our laboratory by a limited number of passages on CEF cells. Amplification of CEF-adapted isolates of IBDV (CEF94 and TY89) was performed by growing freshly prepared chicken embryo fibroblast (CEF) cells in a tissue culture flask (75 cm²) until near confluency. This cell culture was infected with either CEF94 or TY89 (moi = 0.1) and incubated for 48 h at 37° C in a 5% CO₂ incubator. The supernatant of this culture was centrifuged at 6000 g for 15 min. (pelleting

of debris), transferred to clean tubes and subsequently centrifuged at 33.000 g for 3 h. The virus pellet was resuspended in PBS (1% of the initial culture volume). The very virulent IBDV isolate D6948 was propagated in our laboratory in 21-days-old chickens by inoculation of 200 ELD50 (Egg Lethal Doses) per chicken, nasally and by eye-drop. The bursas of Fabricius were collected from the infected chickens three days post infection, and two volumes of tryptose phosphate buffer was added. This mixture was homogenized in a Sorval Omnimixer (3 * 10 sec, maximum speed) and subsequently clarified by centrifugation (6000 g, 10 min). The supernatant was transferred to clean tubes and extracted three times with freon and once with chloroform. Virus preparations were stored at -70° C until further use. QM5 cells (Antin and Ordahl, 1991) were received from the laboratory of R. Duncan (Dalhouse University, Halifax, Nova Scotia, Canada) and maintained by using QT35 medium (Fort Dodge), in a CO₂ incubator (37° C).

Isolation of viral dsRNA

[0041] The genomic dsRNA was purified from the IBDV particles by digesting the viral proteins with Proteinase K (Amresco, 1.0 mg/ml) in the presence of 0.5 % SDS during 2 h at 50° C. The viral dsRNA was purified by phenol/chloroform/isoamylalcohol (25:24:1) extraction (two times) and precipitation with ethanol (2.5V) / NaCl (0.1V, 5M, pH4.8) or with 2 M lithiumchloride (Diaz-Ruiz and Kaper, 1978). The RNA was dissolved in DEPC treated water (10 % of the initial volume) and stored at -20° C until further use. The integrity and purity of the viral RNA was checked on an agarose gel.

Rapid Amplification of cDNA ends.

[0042] The extreme 5'-termini of all genomic RNA strands (the coding and non-coding strands of both the A- and B-segment) of isolate CEF94 were determined. We used 2 ug of genomic dsRNA and 10 pmol of strand- and segment specific primers in a total volume of 12 ul, for each determination. After incubation at 95° C for five min. we transferred this mixture onto ice and added 4 ul of Superscript II first strand syntheses buffer (Gibco/BRL), 2 ul of 100 mM DTT and 2 ul of dNTP's (10 mM each). This mixture was subsequently incubated at 52° C for 2 min, after which 1 ul of reverse transcriptase (Superscript II, Gibco/BRL) was added and incubation at 52° C was continued for one hour. The reverse transcriptase was inactivated by the addition of 1 ul of 0.5 M EDTA. The genomic dsRNA was destroyed by the addition of 2 ul of 6 M NaOH and incubation at 65° C for 30 min. For neutralization, 2 ul of 6 M Acetic acid was added, and cDNA was recovered by using a Qiaex DNA extraction kit (Qiagen) and finally dissolved in 6 ul water. In the anchor ligation reaction we used 2.5 ul of the cDNA preparation, 4 pmol of the anchor, 5 ul T4 ligation buffer and 0.5 ul T4 RNA ligase (New England Biolabs). Incubation was performed at room temperature for 16 h and the reaction was stored at -20° C. To amplify the single stranded cDNA which was ligated to the anchor, we used a nested primer in combination with the anchor primer. The PCR was performed by using the following conditions: 10 pmol of each specific primer, 10 pmol of the anchor primer, 4.5 or 5.5 mM MgCl₂, 1* Taq buffer (Perkin Elmer), 50 uM of each dNTP, 3 units of Taq polymerase (Perkin/Elmer), and 4 ul of the RNA ligation mixture as template, in a total volume of 50 ul. The amplification was performed by 35 cycles through the temperature levels of 92° C (45 sec), 57 or 65°C (45 sec), and 72°C (90 sec). The resulting PCR products were agarose gel purified and digested with *EcoRI* and *XbaI* and ligated (T4 DNA Ligase, Pharmacia), in a pUC18 vector which had previously been digested with the same restriction enzymes. The resulting plasmids were amplified in *E. coli* and nucleotide sequence analysis was performed by using the M13F and M13R primers.

Generation of full length A- and B-segment single stranded cDNA

[0043] To produce full length single stranded cDNA of both the A- and B-segments of CEF94 and D6948, we used a primer specific for the 3'-terminus of the coding strand in the reverse transcription reaction for initiation of the cDNA synthesis. As template we used 1 ug of genomic RNA and 2.5 pmol of ANCI (A-segment specific primer, Table 2) or BNCl (B-segment specific primer, Table 2) in a total volume of 10 ul. After incubation at 98° C for two min. we transferred this mixture immediately onto ice and added 10 ul of RT-mix containing 2* Superscript II first strand syntheses buffer (Gibco/BRL), 20 mM DTT, 2 mM of each dNTP and 100 units of Superscript II (Gibco/BRL). In case of the negative control reaction the addition of Superscript II enzyme was omitted. All tubes were incubated at 50° C for 30 min, after which time 0.5 units of RNase H were added and incubation was continued at 37° C for 15 min. Water (80 ul) was added to each tube, and dsRNA and cDNA was purified by a phenol/chloroform/isoamylalcohol (25:24:1) extraction and precipitated by using a standard ethanol/NaAc precipitation protocol. Obtained pellets were dissolved in 20 ul of water and stored at -20° C.

Amplification of full length cDNA using a PCR based protocol

[0044] The full length single stranded cDNA of both the A-and B-segment were amplified by using PCR. The primers which hybridize to the 3'-terminus of the non-coding strand of the A-segment (T7AC0, Table 2) and B-segment (T7BC1, Table 2) both have a non-hybridizing 5' extension of 24 nt containing a T7 promoter sequence and an EcoRI site. The primers that hybridize to the 5' terminus of the coding strand of the A-segment (ANCO, Table 2) and B-segment (BNC1, Table 2) match exactly. As template we used 5 ul of the above mentioned RT reaction and the PCR was performed in the presence of 1* Expand High Fidelity buffer, 50 uM of each dNTP, 0.2 pmol of each primer, 1.5 units of Expand High Fidelity enzyme, and 2.0 mM MgCl₂ (A-segment) or 4.0 mM MgCl₂ (B-segment). Amplification was performed by cycling 35 times between 94° C (15 sec), 58° C (15 sec) and 72° C (5 min) in case of A-segment amplification (A-program) and cycling for 35 times between 94° C (15 sec), 54° C (15 sec) and 72° C (5 min) in cases of B-segment amplification (B-program), using a Biometra T3 thermocycler. The yield of PCR products was checked on a 1.0% agarose gel.

Cloning and analysis of the generated PCR fragments

[0045] The full length PCR fragments which were generated three times independently from genomic dsRNA, were isolated from the agarose gel by using a Qiaex gel purification kit (Qiagen) and ligated in the pGEM-Teasy (Promega) vector according to the suppliers instructions. The ligated plasmids were used to transform *E. coli* DH5-alpha cells which were subsequently grown under ampicillin selection (100 ug/ml) and in the presence of IPTG (0.8 mg per petri-dish) and BluO-gal (0.8 mg per petri-dish). Plasmid DNA of white colonies was prepared and analyzed by restriction enzyme digestion and agarose gel separation. The nucleotide sequences of the cloned cDNA's were determined by using a ABI310 automated sequencer and A- and B-segment specific primers. The consensus nucleotide sequences of both segments of CEF94 and of both segments of D6948 were determined (Fig. 2) and the corresponding amino acid sequence of the open reading frames was deduced (Fig. 3). By using the cDNA of two independent clones we restored one amino acid mutation present in the A-segment clone (V542A), resulting in pHB-36W, one amino acid mutation in the A-segment clone of D6948 (P677L), resulting in pHB-60, and one amino acid mutation in the B-segment of D6948 (Q291X), resulting in pHB-55. No amino acid mutations were present in the B-segment cDNA clone of CEF94 (pHB-34Z).

Introduction of a Hepatitis Delta Virus ribozym

[0046] The Hepatitis Delta Virus ribozym was first introduced into the *E. coli* high copy number plasmid pUC-18 by digesting transcription vector 2.0 (Pattnaik et al., 1992) with restriction enzymes *Xba*I and *Sma*I. The resulting 236 bp fragment, which contains the Hepatitis Delta Virus Ribozym and a T7 RNA polymerase terminator, was ligated in the pUC18 vector which previously was digested with *Xba*I and *Sma*I, yielding pUC-Ribo. Plasmids containing the A- and B-segment of CEF94 and D6948 were used as template in a full length PCR using the above described conditions, and primers specific for either the A-segment (T7AC0 and ANCO) and B-segment (T7BC1 and BNC1). The PCR fragments were agarose gel purified (Qiaex), blunt-ended by using T4 DNA polymerase, and subsequently digested with *Eco*RI. The resulting DNA fragments were directionally cloned into the pUC-Ribo vector which previously had been digested with *Sma*I and *Eco*RI. The resulting plasmids were used as template in an *in vitro* transcription-translation reaction (TnT-T7Quick, Promega). The autoradiogram of SDS-PAGE analyses of the translation products revealed three dominant bands pVP2 (48-49 kDa), VP3 (32-33 kDa), and VP4 (28-29 kDa) when pHB-36A (A-segment of CEF94) or pHB-60 (A-segment of D6948) was used as template. One dominant band (VP1 (91 kDa)) was found when we used plasmid pHB-34Z (B-segment of CEF94) or pHB-55 (B-segment of D6948) as template (data not shown).

Introduction of a genetic tag

[0047] To distinguish infectious virus generated from cloned cDNA from wild-type virus we introduced a genetic tag in the 3'-UTR of the A-segment of IBDV-A isolate. Two nucleotides of pHB-36A were mutated (C3172T and 3T173A) thereby introducing a unique *Kpn*I restriction site (GGTAAC). These mutations were introduced by the method described by Higuchi (1990). A 383 bp fragment of the resulting PCR fragment was ligated (Rapid ligation kit, Boehringer Mannheim) into the full length A-segment clone (pHB-36A) by using two unique restriction sites (*Bgl*II and *B*lpl). The resulting plasmid pHB-36W was amplified in *E. coli*. The genetic tag was present in this full length CEF94 A-segment clone, as could be concluded from sequence analysis and digestion with restriction enzyme *Kpn*I (data not shown). No difference was observed in the resulting protein pattern when either pHB-36A or pHB-36W was used as template in an *in vitro* transcription/translation reaction (data not shown).

Construction of mosaic A-segment cDNA

[0048] We constructed plasmids containing mosaic IBDV A-segments which partly consisted of cDNA of one isolate (CEF94) and partly of cDNA of another isolate (D6948). To construct these plasmids we have amplified specific parts of cDNA using appropriate IBDV specific or selective primers. The amplified PCR fragment of cDNA of D6948 was subsequently used to replace the corresponding part in plasmids pHB-36W, using restriction endonucleases and T4 DNA ligase (Rapid DNA Ligation, Boehringer Mannheim).

[0049] For the construction of pHB36-vvVP2 (exchange of pVP2 encoding part; Table 4) we have used IBDV specific to generate the mosaic PCR-VP2D fragment (2256 bp, see Fig. 5a). The internal part of this PCR fragment was used to exchange the corresponding part of pHB-36W, using unique sites for restriction enzymes *EcoRI* and *SacI*. For the construction of plasmid pHB36-vvVP3 (Table 4) we used IBDV specific primers to generate a mosaic PCR-VP3c fragment (2154 bp, see Fig. 5b). The internal part of this PCR fragment was used to exchange the corresponding part of pHB-36W, using unique sites for the *EagI* and *KpnI* (genetic tag site) restriction enzymes.

For the construction of plasmid pHB36-vvVP4 (Table 4) we used IBDV specific primers to generate a mosaic PCR-VP4d fragment (2154 bp, see Fig. 5c). The internal part of this PCR fragment was used to exchange the corresponding part of pHB-36W, using the unique site for restriction enzymes for *EagI* and *DraIII*.

[0050] plasmids pHB36-vvVP2, -vvVP3, and -vvVP4 were partly analysed by nucleotide sequence determination to conform that no unintended mutations were introduced during the described manipulations.

Introduction of a serological marker

[0051] To obtain the cDNA of the A-segment of a serotype II IBDV isolate we generated single stranded cDNA of TY89 as described above, by using the ANC1 primer. The coding region of the VP3 protein was subsequently three times independently amplified in a PCR by using 2 ml of RT-material, 1* Taq buffer, 50 uM of each dNTP, two IBDV serotype II specific primers (0.2 pMol each), 1.5 units of enzyme, and 3.0 mM $MgCl_2$ in a 0.1 ml reaction volume. Amplification was performed by cycling 35 times between 94° C (15 sec), 52° C (15 sec) and 72° C (1 min). The resulting 956 bp fragment was cloned in the pGEM-TEasy vector and the consensus nucleotide sequence was determined (Fig. 2a). One of the isolated plasmids contained the TY89 VP3 consensus sequence (pSV-VP3-TY89, Fig. 4) and was used as template to generate a 893 bp PCR fragment (see Fig. 5d). This PCR fragment was subsequently used to replace the corresponding part of plasmid pHB36W-vvVP3, by using the artificially introduced *KpnI* (nt 3175) and *SacII* (nt 1760) restriction sites in both plasmid pSV-VP3-TY89 and pHB36W-vvVP3. The resulting plasmid (pHB36-vvVP3, see Fig. 5d) encodes the N-terminal 722 amino acids of the CEF94 polyprotein and the 290 C-terminal amino acids of the TY89 polyprotein. The intended exchange was confirmed by nucleotide sequence analysis.

[0052] The same approach was used to exchange the C-terminal half of the coding sequence of the VP3 protein. In stead of the artificially introduced *SacII* site, we made use of the *Scal* (nt 2799) site which is naturally present both in the TY89 and in the CEF94 cDNA of the A-segment, in combination with the artificially introduced *KpnI* site (nt 3172). The resulting plasmid (pHB36-s2VP3C, Table 4) encodes a polyprotein consisting of the N-terminal 890 amino acid of the CEF94 polyprotein, in combination with the C-terminal 122 amino acids of the TY89 polyprotein.

[0053] For the construction of plasmid pHB36-s2VP3N (see Table 4) we have replaced the *Scal* (nt 2799) - *KpnI* (nt 3172) part of plasmid pHB36-s2VP3 with the corresponding part of plasmid pHB-36W. Using the specific restriction endonucleases *Scal* and *KpnI*, and T4 ligase. The nucleotide sequence of plasmid pHB36-s2VP3N was conformed by sequence analysis.

[0054] For the introduction of the C-terminal encoding part of the VP3 protein of IBDV isolate TY89 into the cDNA of isolate D6948 we have exchanged part of plasmid pHB-60 (nt 1760 -> nt 3260) with the corresponding part of plasmid pHB36-s2VP3C. Plasmid pHB36-s2VP3C was digested with restriction enzymes *SacII* and *XbaI* and a 1735 bp fragment was recovered from an agarose gel by Qiaex gel extraction kit (Qiaex). This DNA fragment was ligated in the 4440 bp vector fragment of pHB-60 which had previously been digested with the same restriction enzymes. The resulting plasmid (pHB60-s2VP3C1, Table 4) contains cDNA derived from IBDV isolate D6948 (nt 1 to 1760), CEF94 (nt 1760 to 2799 and nt 3175 to 3260), and TY89 (nt 2799 to 3175).

Transfection of QM5 cells

[0055] QM5 cells, grown to 80% confluency in 60 mm dishes, were infected with Fowl Pox T7 (FPT7) (Britton et al., 1996) one hour prior to transfection. FPT7 infected QM5 cells were subsequently washed once with 5 ml QT-35 medium and incubated with 2 ml fresh Optimem 1 (Gibco/BRL) two times during 15 min. In the mean time, DNA (2.0 to 4.0 ug) was incubated in 0.5 ml Optimem 1 supplemented with 25 ul LipofectAMINE (Gibco/BRL) and kept at room temperature for at least 30 min. The washed QM5 cells were covered with 4 ml of Optimem 1, the DNA/LipofectAMINE transfection mixture was added and the cells were stored for 18h in a 5.0% CO_2 incubator at 37°C.

Detection of recombinant IBDV after transfection of QM5 cells

[0056] Transfected QM5 cells were washed once with PBS after the transfection. Infectious recombinant IBDV (rIBDV) was recovered from transfected QM5 cells by covering them with 4 ml of QT-35 medium supplemented with 5% fetal calf serum and 2% of an antibiotic mix (1000 U/ml Penicillin, 1000 ug/ml Streptomycin, 20 ug/ml Fungizone, 500 ug/ml; Polymixin B, and 10 mg/ml Kanamycin) and incubation for 24 h at 37° C (5.0% CO₂). The supernatant was filtered through a 200 mM filter (Acrodisc) to remove FPT7 virus and was subsequently stored at -70° C or used directly for quantitation of rIBDV. Recombinant mosaic IBDV (mIBDV) which contains at least the pVP2 from vvIBDV isolate D6948 is unable to re-infect QM5 cells (see Table 5). Therefore, supernatant of transfection experiments which contained D6948 pVP2 encoding cDNA were used to infect 11-days-old, embryonated eggs via the chorioallantoic membrane (CAM) route. To determine the presence of infectious IBDV, the embryo's were collected five days post-infection, homogenized by using a Sorval Omnimixer (3 * 10 sec, max. speed) and assayed for the presence of IBDV proteins in a IBDV protein specific Elisa.

Serological differentiation of recombinant mosaic IBDV (mIBDV)

[0057] Different monoclonal antibodies were used to detect recombinant mosaic IBDV (mIBDV) that contained part of the TY89 VP3 or the complete TY89 VP3. The mIBDV's were used to infect QM5 or primary bursa cells and incubated for 24h (QM5 cells) or 48h (primary bursa cells) in a 5% CO₂ incubator at 37° C or 39° C, respectively. The infected cells were subsequently fixed and an immunoperoxidase monolayer assay (IPMA) was performed by using monoclonal antibodies which are either specific for VP2 of serotype I IBDV (Mab 1.4), or specific for VP3 of serotype II (Mab T75), or specific for VP3 of serotype I (Mab B-10 or C-3).

Virulence of rIBDV in young SPF chickens

[0058] To evaluate the degree of virulence of the generated rIBDV, srIBDV, and mIBDV isolates we have inoculated 12 groups (10 21-days old SPF chickens) with these viruses. Each chicken received nasally and by eye-drop 1000 ELD50 IBDV, with exception with the negative control group, which received only PBS. The animals were monitored for clinical signs and dead chicks were removed each day. At 9 days post infection, all the chicks from the negative control groups and all the surviving chicks from groups in which mortality had occurred, were bled (5 ml) and euthanized for necropsy. From the other groups, 6 chicks were bled (5 ml) and taken for necropsy at day 9 post infection, where as the remaining 4 were bled (5 ml) and taken for necropsy at day 15 post infection. Bursa and body weight was determined of all chicks which had been euthanized at day 9 post infection

Results

Nucleotide sequence determination of the 5'-termini.

[0059] One group has reported the 5'- and 3'-terminal sequences of the segmented dsRNA genome of IBDV (Mundt and Muller, 1995). To verify the terminal sequence of the genome of IBDV and to be able to produce the exact cDNA sequence of a single IBDV isolate we have determined the 5' terminal sequences of both the coding and non-coding strands of the two genomic segments of CEF94, a Chicken Embryo Fibroblast (CEF) adapted, classical isolate of IBDV, by using the RACE (Rapid Amplification of cDNA Ends) technique (Frohman et al., 1988). The RACE analysis was performed in duplicate on each of the four 5'-termini of the CEF94 genome. The resulting sequence data (Table 3) show that the length of the 5'-termini of the coding strands was the same in all cases. Furthermore we found that the nucleotide sequence was identical, except for the last nucleotide which varied in a few clones. This is in contrast to the sequence data of the 5'-termini of the non-coding strands, which varied in length considerably. We also found that the last nucleotide, although preferably a cytosine, varied in some clones similarly to what we found for the 5'-termini of the coding strands. The consensus sequence for the 3'-terminal nucleotide of the A-segment coding strand of CEF94 differs from the nucleotide sequence reported by Mundt and Muller (Mundt and Muller, 1995), i.e. being a cytosine in stead of a thymine.

Generation of plasmids containing full length IBDV cDNA

[0060] Using the sequence data of the 5'-termini we cloned the entire coding and non-coding cDNA sequences of the A-segment and B-segment of classical isolate CEF94 by means of RT-PCR. Using the same procedure and using the same primers we also generated the entire coding and non-coding cDNA of the A- and B-segment of a non-CEF-adapted, very virulent IBDV isolate D6948. The nucleotide sequence of the entire genome of both isolates was deter-

mined three times independently. This sequence information enabled us to generate a consensus nucleotide sequence of both the A- and B-segments of IBDV isolates CEF94 and D6948 (Fig. 2A).

Fowlpox T7 polymerase expression system

[0061] One system for generating infectious IBDV virus using *in vitro* synthesized mRNA derived from cDNA of a CEF-adapted IBDV isolate has previously been described (Mundt and Vakharia, 1996). This system is based upon *in vitro* run-off transcription from the T7 promoter which was artificially introduced in front of the cDNA sequences of the A- and B-segments. This RNA is subsequently transfected into VERO cells, after which infectious IBDV virus could be harvested from these cells. One of the drawbacks of this system is that the *in vitro* generated RNA has to contain a 3'-G-ppp5'- (cap structure) on its 5'-end in order to get translation of the introduced RNA into the viral proteins, and hence replication of viral RNA. The *in vitro* production of high quality mRNA is both inefficient and expensive as a cap structure has to be present at the 5'-end. Furthermore, expression levels from transfected RNA are generally low due to the short half-life of RNA. To circumvent the drawbacks of generating *in vitro* capped RNA and low expression levels, we have explored the possibility of using an *in vivo* based T7-expression system (Fowlpox T7 polymerase expression system, Britton et al., 1996) for generation of viral RNA from plasmids containing full length IBDV cDNA.

Generating of infectious IBDV using Fowlpox infected cells

[0062] To be able to generate IBDV from cloned cDNA which has the authentic terminal sequences, we introduced the cis-acting Hepatitis Delta Virus (HDV) ribozym (Chowrira et al., 1994) downstream of the cDNA sequence of the A- and B-segments (Fig. 4). Furthermore we introduced an additional modification in 3' untranslated region of the CEF94 A-segment. By exchanging 2 nucleotides we introduced a unique *KpnI* endonuclease restriction site in this cDNA. The introduction of this unique restriction site enables us to distinguish between wild-type IBDV and infectious IBDV virus generated from cloned cDNA (genetically tagged rIBDV). As expected, this plasmid yields the same viral proteins in an *in vitro* transcription-translation reaction as the A-segment clone without the genetic tag (data not shown). Plasmid pHB-36W (A-segment CEF94), pHB-60 (A-segment D6948), pHB-34Z (B-segment CEF94), and pHB-55 (B-segment D6948) were used individually to transfect FPT7 infected QM5 cells as described in the Materials and Method section. To analyze whether the transfected QM5 cells expressed IBDV proteins, we performed an IPMA, 24 h after transfection. We used polyclonal antiserum directed either against VP3 (pHB-36W and pHB-60 transfections) or VP1 (pHB-34Z and pHB-55 transfections) in this analysis. About 10 to 50% of the QM5 cell expressed VP3 after transfection with pHB-36W or pHB-60 (data not shown). When B-segment encoding plasmids were used (pHB-34Z or pHB-55) we found that the same percentage of cells (about 10 to 50%) were expressing VP1 (data not shown). Subsequently, we co-transfected combinations of plasmids containing the A- and B-segment cDNA's into FPT7 infected QM5 cells. To screen for infectious recombinant IBDV (rIBDV) in the supernatant of the transfected QM5 cells, we transferred part of the supernatant (10% of the volume) after 18 h onto fresh QM5 cells or onto primary bursa cells. We only could detect rIBDV when A-segments plasmids in combination with B-segments plasmids were used to transfect the QM5 cells. rIBDV could not be detected when supernatant of the cells transfected with A-segment (pHB-60) and B-segment (pHB-55) of D6948 (rD6948) was transferred onto QM5 cells. However, when the co-transfection supernatant of pHB-60 and pHB-55 was transferred onto primary bursa cells or embryonated eggs we were able to show the presence of infectious IBDV (rD6948) in primary bursa cells (after 48h) and in embryonated eggs (after five days). The presence of rIBDV in the first passage was established by using either an IPMA (QM5 cells or primary bursa cells) or an IBDV specific Elisa (embryonated eggs). The generated rCEF94 and rD6948 isolates were amplified in 10-days old embryonated SPF eggs and subsequently used to infect 21-days old SPF chickens (10 chickens per IBDV isolate). The resulting data of the animal experiment (Table 6) shows that the mortality, body weight, bursa weight, and bursa-body weight ratio, caused by rD6948 are the same as the parent very virulent D6948 isolate. Also at necropsy, gross lesions of bursa were as severe for rD6948 as for the parental D6948 isolate (data not shown). From this chicken experiment it is concluded that rD6948 has retained the properties of a very virulent IBDV isolate, and is truly a very virulent rIBDV.

Detection of the genetic tag

[0063] Supernatant of rCEF94 infected QM5 cells was harvested and IBDV was isolated by centrifugation as described in the material section. The dsRNA genome was extracted and an A-segment specific primer was used to generate single stranded cDNA, by using reverse transcriptase. The cDNA was subsequently amplified by PCR. The generated PCR fragment was cloned into a high copy number *E. coli* plasmid (pGEM-Teasy, Promega) and was either digested with *KpnI* or used for nucleotide sequence determination. The presence of the genetic tag in rCEF94 was confirmed in both analyses.

Identification of a lethal amino acid mutation in VP4

[0064] Plasmid pHB-36 (A-segment CEF94, Table 4) contained a single nucleotide substitution at position 1875 (thymine instead of a cytosine) compared to the consensus CEF94 A-segment sequence (Fig. 2A). This nucleotide substitution leads to a valine at position 582 of the polyprotein instead of an alanine, which is encoded by the consensus sequence (V582A, Fig. 3A). As this amino acid mutation is present in the viral protease (VP4), we subsequently checked whether this protease was still able to autocatalytically liberate the viral proteins (pVP2, VP3 and VP4) from the polyprotein. When plasmid-pHB-36 was used as template in a coupled in vitro transcription/translation reaction in the presence of ³⁵S labeled methionine we found a delayed splicing of the polyprotein (data not shown). Apart from the viral proteins which are found in case of normally spliced polyprotein (pVP2; VP3 and VP4), we found intermediate spliced products (60 kDa: VP4+VP3), and non-spliced polyprotein (data not shown). Although the viral protease (VP4) of clone pHB-36 is able to liberate the structural viral proteins (pVP2 and VP3) from the polyprotein, this clone did not yield rIBDV when using the FPT7 based transfection protocol as described above. Rapid autocatalytic cleavage of the polyprotein is apparently necessary for the generation of infectious rIBDV. We expect that other mutations within VP4 which alter the rate or specificity of the autocatalytic cleavage of the polyprotein will also have a negative effect on viability of the generated rIBDV. Furthermore mutations in the region of the cleavage sites (pVP2-VP4 and VP4-VP3) may also have a negative effect on replication of rIBDV. Any mutation, introduced by modern molecular biological techniques into the cDNA of a very virulent IBDV may enable us to generate rIBDV which has a reduced viability and which can be used as a live or killed IBDV vaccine.

Generation of segment reassortant IBDV

[0065] Transfection of CEF94 A-segment cDNA (pHB-36W) in combination with D6948 B-segment cDNA (pHB-55) yielded segment reassorted IBDV (srIBDV-CADB) when supernatant of QM5 transfected cells was transferred onto fresh QM5 cells (Table 5). When D6948 A-segment cDNA (pHB-60) was used in combination with CEF94 B-segment cDNA (pHB-34Z) no infectious srIBDV (srIBDV-DACB) could be detected on QM5 cells (Table 5). However, when primary bursa cells were used to assay for the presence of infectious IBDV we found in both cases (srIBDV-CADB and srIBDV-DACB) infected cells after 24h of incubation. Out of the population of primary bursa cells, only lymphoid cells were infected with srIBDV-DACB, while both lymphoid and fibroblast cells were infected in the case of srIBDV-CADB. The srIBDV-DACB isolate induces the same clinical signs as D6948, while the srIBDV-CADB isolate has a comparable virulence as CEF94 (Table 6).

Construction of mosaic IBDV

[0066] By using modern molecular biological techniques such as those described above, we have created mosaic recombinant IBDV (mIBDV) which exists partly of cDNA derived from CEF94, and partly from D6948 (vvIBDV) or TY89 (a serotype II IBDV isolate). Replacement of the pVP2 protein encoding sequence of CEF94 by the corresponding part of the D6948 yielded only virus (mCEF94-vvVP2) when the supernatant of transfected cells was transferred to cells which are normally susceptible for non CEF-adapted vvIBDV, i.e. primary bursa cells or embryonated eggs. (Table 5). Replacement of the VP3 or VP4 protein encoding sequence of CEF94 with the corresponding part of D6948 yielded mIBDV by using QM5 cells as recipient in the first passage (mCEF94-vvVP3 and mCEF94-vvVP4 respectively).

[0067] Replacement of the complete VP3 cDNA (290 amino acids) of CEF94 by the corresponding part of the TY89 cDNA yielded a plasmid which encoded a polyprotein consisting of pVP2 and VP4 derived from CEF94 and of VP3 derived from TY89. When this plasmid (pHB36-s2VP3) was used in an in vitro transcription-translation reaction, all the expected proteins, pVP2, VP4 and VP3 were present (data not shown). Transfection of this plasmid in combination with a plasmid (pHB-34Z) containing the B-segment cDNA of CEF94 yielded a mosaic IBDV (mCEF94-s2VP3). Two monoclonal antibodies which are specific for serotype I VP3 (Mab B10 and C3) were unable to recognize this mCEF94-s2VP3, while an antibody which is specific for the serotype II VP3 (Mab T75) did recognize this mosaic virus (Table 5). As expected the mCEF94-s2VP3 was also recognized by a serotype I specific, neutralizing monoclonal antibody directed against VP2 of the CEF94 isolate (Mab 1.4). The TCID₅₀ on QM5 cells, which was determined 18 hours after transfection, was considerably lower (3 logs) in the case of mCEF94-s2VP3 compared to rCEF94. Furthermore we found that only single QM5 cells were infected by mCEF94-s2VP3 after 24 h. This is in contrast to the plaque forming phenotype of CEF94 and rCEF94 on QM5 cells after 24 h of infection. To generate mIBDV which has the same replication and plaque forming characteristics as rCEF94, but which is still antigenetically different from rCEF94 we subsequently exchanged only the N-terminal part (168 amino acids) or C-terminal part (122 amino acids) of the VP3 of CEF94 by the corresponding sequence of TY89. When these mosaic A-segment plasmid (pHB36-s2VP3N or pHB36-s2VP3C) were transfected in combination with pHB-34Z (CEF94 B-segment) we obtained mosaic IBDVs (mCEF94-s2VP3N or mCEF94-s2VP3C) with replication capabilities in QM5 cells that are equal (mCEF94-s2VP3C)

or slightly reduced (mCEF94-s2VP3N) to those of rCEF94 IBDV (data not shown). Subsequently we checked the recognition of mCEF94-s2VP3N and mCEF94-s2VP3C virus by several Mabs in an IPMA on QM5 infected cells (Table 5). Mab T75 which is specific for VP3 of serotype II also recognized mCEF94-s2VP3C, while the recognition of mCEF94-s2VP3N was slightly reduced. Mab B10, which is specific for VP3 of serotype I did not recognize rCEF94-s2VP3C, while it still recognized mCEF94-s2VP3N. Another Mab (C3) which did not react with mCEF94-s2VP3 infected cells did react with mCEF94-s2VP3C infected cells, although the reaction was reduced compared to QM5 cells infected with rCEF94 (Table 5) mCEF94-s2VP3N was not recognized by Mab C3. The serotype I specific, neutralizing antibody Mab 1.4 which recognizes VP2 recognized, as expected, both mCEF94-s2VP3N and mCEF94-s2VP3C.

[0068] The coding sequence of the C-terminal part of serotype II VP3 (122 amino acids) was also used to replace the corresponding part of the cDNA of D6948. During the exchange we have replaced some D6948 cDNA sequence (encoding for C-terminal part of VP4 and the N-terminal part of VP3, and the 3'-UTR) with the corresponding sequence of CEF-94 (see Fig. 5g). The resulting plasmid (pHB60-s2VP3C1) was, together with pHB-55 (B-segment D6948), transfected into FPT7 infected QM5 cells. Supernatant of these transfected QM5 cells was collected after 24 h and was transferred to embryonated eggs and primary bursa cells. By using monoclonal antibodies we were able to detect infected cells in the monolayer of primary bursa derived cells (see Table 5). mD6948-s2VP3C1 gave the same reaction pattern with the monoclonal antibodies as mCEF-s2VP3C did. Isolate mD6948-s2VP3C1 (1000 ELD50/chicken) was also used to infect 10 SPF chickens (21-days old) to evaluate its virulence. This mIBDV isolate did not cause any mortality in a 9-days course, opposite to the D6948, rD6948 and srlBDV-DACB isolates (Table 6). However, the bursa is severely damaged by this mIBDV, as the bursa-body weight ratio of this group is same as found in the groups which received D6948 or rD6948. This indicates that mD6948-s2VP3C1 is still able to replicate and induce apoptosis in the bursa of Fabricius.

LEGENDS TO FIGURES

[0069]

Fig. 1: Antibody titers in broilers having high levels of maternal antibody at day 0.

Fig. 2a: Nucleotide sequences A-segments

Fig. 2b: Nucleotide sequences B-segments

Fig. 3a: Amino acid sequences polyproteins

Fig. 3b: Amino acid sequence VP1

Fig. 3c: Amino acid sequence VP5

Fig. 4: Plasmid drawings

Fig. 5a: Construction of pHB36-vvVP2

Fig. 5b: Construction of pHB36-vvVP3

Fig. 5c: Construction of pHB36-vvVP4

Fig. 5d: Construction of pHB36-s2VP3

Fig. 5e: Construction of pHB36-s2VP3C

Fig. 5f: Construction of pHB36-s2VP3N

Fig. 5g: Construction of pHB60-s2VP3C1

Table 1:

Classification of live IBDV vaccines used to induce active protection in young chickens which are passively protected by maternal IBDV antibodies.		
Type of vaccine (live IBD virus)	Ability to induced an immune response when IBDV antibody titers are equal or below	Immunosuppressive
Mild	50-100	No
Intermediate	100-200 *	No
Strong	500 *	Yes

* The Animal Health Service (Deventer, The Netherlands) uses an Idexx Elisa value of 128 (2log7) as the maximum titer for the use of live intermediate vaccines and a value of 512 (2log9) for strong vaccines.

Table 2 Primers (oligonucleotides) used for sequence determination, in RT-PCR or PCR reactions. Nucleotides which are unable to hybridize with wild-type IBDV genomes are given in small face. Primers which are specific either for the serotype II (s2) or very virulent (vv) genome are indicated.

Name	Sequence	Position
Anchor	cacgaattcactatcgattctggatccttc	-
Anchor Primer	gaaggatccagaatcgatag	-
ANC0	GGGGACCCGCGAACGGATC	A: -1/-18
ANC1	GGGGACCCGCGAACGG	A: -1/-16
T7AC0	ggaattctaatacgactcactataGGATACGATCGGTCTGACCCCGG	A: 1/23
BNC1	GGGGGCCCCCGCAGG	B: -1/-15
T7BC1	ggaattctaatacgactcactataGGATACGATGGGTCTGACCCT	B: 1/21

Table 3 Nucleotide sequence corresponding to the 5'- and 3'-termini of the coding strands of the two genomic segments of IBDV (CEF94). Numbers behind specific sequences indicate the number of times each sequence was obtained.

5'-terminus of the A-segment coding strand	Complementary sequence of the 5'-terminus of the B-segment coding strand	Complementary sequence of the 5'-terminus of the B-segment non-coding strand
5'UGAUACGAUC>>>>	5'UGAUACGAUC>>>> (2x)	>>>GGGGGCCA ³
5'AGAUACGAUC>>>>	5'GGAUACGAUC>>>> (5x)	>>>GGGGGCCU ³
5'GGAUACGAUC>>>> (7x)		>>>GGGGGCCC ³ (2x)
		>>>GGGGGCCC ³ (2x)
		>>>GGUGGCCCCC ³
		>>>GGGGGCCCCC ³
		>>>GGGGGCCCCC ³
Consensus 5'GGAUACGAUC>>>>	>>>CGGGUCCCC ³ (nt 3260) 5'GGAUACGAUC>>>>	>>>GGGGGCCCCC ³ (nt 2827)

Table 4 Description of the used plasmids

Name	Based on plasmid	Description
pUC18-Ribo	pUC18	Contains the <i>SmaI-XbaI</i> fragment of pTV-2A
pHB-36A	pUC18-Ribo	Contains the consensus cDNA sequence of the A-segment of CEF94 (see Fig. 2a)
pHB-36W	pHB-36A	An artificially introduced <i>KpnI</i> -site (genetic tag) in the 3'-UTR of the CEF94 A-segment encoding cDNA (Fig. 2)
pHB-36	pHB-36A	Contains a lethal amino acid substitution in the VP4 part of the polyprotein (V582A)
pHB-60	pUC18-Ribo	Contains the consensus cDNA sequence of the D6948 A-segment (see Fig. 2a)
pHB-34Z	pUC18-Ribo	Contains the consensus cDNA sequence of the CEF94 B-segment (see Fig. 2b)
pHB-55	pUC18-Ribo	Contains the consensus cDNA sequence of the D6948 B-segment (see Fig. 2b)
pSV-TY89-VP3	pGEM-Teasy	Contains the consensus cDNA of TY89 encoding the entire VP3 (A-segment, see Fig. 2)
pHB36-vvVP2	pHB-36W	Contains D6948 A-segment cDNA which encodes the entire VP2 (453 amino acids)
pHB36-vvVP3	pHB-36W	Contains D6948 A-segment cDNA which encodes the entire VP3 (289 amino acids)
pHB36-vvVP4	pHB-36W	Contains D6948 A-segment cDNA which encodes the entire VP4 (270 amino acids)
pHB36-s2VP3	pHB-36W	Contains TY89 A-segment cDNA which encodes the entire VP3 (289 amino acids)
pHB36-s2VP3C	pHB-36W	Contains TY89 A-segment cDNA which encodes the C-terminal part (122 amino acids) of VP3
pHB36-s2VP3N	pHB-36W	Contains TY89 A-segment cDNA which encodes the N-terminal part (168 amino acids) of VP3
pHB60-s2VP3C1	pHB-60	Contains cDNA encoding a mosaic polyprotein (D6948 (1-543 AA), CEF94 (544-889 AA), and TY89 (890-1012 AA). The 5'-UTR is derived from D6948, while the 3'-UTR is derived from CEF94. An unique <i>KpnI</i> -site (genetic tag) is furthermore present in the 3'-UTR

Table 5 Description of the generated rIBDV, srIBDV, and mIBDV. The ability of these viruses to infect QM5 or primary bursa cells was examined in an immuno peroxidase monolayer assay (IPMA) using either polyclonal serum directed against VP3 or monoclonal antibodies directed against VP2 of IBDV serotype I (1.4), VP3 of serotype II (T-75), or VP3 of serotype I (B10 and C3); nd means not determined.

IBDV virus	Derived from plasmids		Replication on			
	A-segment	B-segment	QM5 cells	Bursa	Mab's	
rCEF94	pHB-36W	pHB-34Z	+	+	1.4 +	B10 + C3 +
rD6948	pHB-60	pHB-55	-	+	+	+
srIBDV-DACB	pHB-60	pHB-34Z	-	+	nd	nd
srIBDV-CADB	pHB-36W	pHB-55	+	nd	nd	nd
mCEF94-vvVP2	pHB36-vvVP2	pHB-34Z	-	+	nd	nd
mCEF94-vvVP3	pHB36-vvVP3	pHB-34Z	+	nd	nd	nd
mCEF94-vvVP4	pHB36-vvVP4	pHB-34Z	+	nd	nd	nd
mCEF94-s2VP3	pHB36-s2VP3	pHB-34Z	+	nd	+	+/-
mCEF94-s2VP3C	pHB36-s2VP3C	pHB-34Z	+	nd	+	+/-
mCEF94-s2VP3N	pHB36-s2VP3N	pHB-34Z	+	nd	+/-	-
MD6948-s2VP3C1	pHB60-s2VP3C1	pHB-55	-	+	+	+/-

Table 6 Clinical data of 21-day old chickens infected with wild-type, rIBDV, srIBDV or mIBDV isolates (12 groups of 10 chickens). Each chicken was inoculated with 1000 ELD₅₀ IBDV, except for the negative control group (PBS), and each group was kept in a separate isolator. The bursa and body weight of euthanized chickens was determined at nine days post infection. Standard deviation is given between brackets, together with the number of animals (n) used for determination of the given numbers. The bursa/body weight ratio for each animal was calculated and mean values (standard deviation) per group are given.

IBDV virus	Number of deads (after 9 days)	Body weight (grams)	Bursa weight (grams)	Bursa/Body weight (*1000)
PBS	0	305 (29, n=10)	1.9 (0.4, n=10)	6.1 (1.2)
CEF94	0	341 (16, n=6)	2.0 (0.6, n=6)	6.0 (1.8)
D6948	3	245 (56, n=7)	0.4 (0.1, n=7)	1.7 (0.6)
rCEF94	0	317 (15, n=6)	1.3 (0.5, n=6)	4.2 (1.3)
rD6948	5	261 (24, n=5)	0.4 (0.1, n=5)	1.7 (0.2)
srIBDV-DACB	2	263 (35, n=8)	0.4 (0.1, n=8)	1.5 (0.3)
srIBDV-CADB	0	314 (13, n=6)	1.8 (0.8, n=6)	5.7 (2.7)
mCEF94-vvVP2	0	309 (27, n=6)	0.6 (0.2, n=6)	1.9 (0.4)
mCEF94-vvVP3	0	325 (33, n=6)	2.0 (0.3, n=6)	6.2 (0.7)
mCEF94-vvVP4	0	330 (23, n=6)	1.5 (0.5, n=6)	4.4 (1.3)
mCEF94-s2VP3C	0	320 (11, n=6)	1.3 (0.4, n=6)	4.1 (1.3)
mD6948-s2VP3CI	0	315 (26, n=6)	0.6 (0.2, n=6)	1.9 (0.6)

Table 7: Origin and phenotype of the IBDV isolates

Isolate	Reference	Virulence
D6948	Boot et al., unpublished	Very virulent
rD6948	Boot et al., unpublished	Very virulent
UK661	Brown and Skinner, 1996	Very virulent
5123	Ter Huurne et al., unpublished	Very virulent
96-B4	Ter Huurne et al., unpublished	Avirulent
96-C4	Ter Huurne et al., unpublished	Avirulent
96-C5	Ter Huurne et al., unpublished	Avirulent
96-C6	Ter Huurne et al., unpublished	Very virulent
97-B3	Ter Huurne et al., unpublished	Avirulent
97-B4	Ter Huurne et al., unpublished	Very virulent
97-B5	Ter Huurne et al., unpublished	Very virulent
97-B6	Ter Huurne et al., unpublished	Very virulent
Zoontjes	Ter Huurne et al., unpublished	Very virulent
Hungary	Ter Huurne et al., unpublished	Very virulent
OKYM	Yamaguchi et al., 1996	Very virulent
OKYMT	Yamaguchi et al., 1996	Avirulent
TKSM	Yamaguchi et al., 1996	Very virulent
TKSMT	Yamaguchi et al., 1996	Avirulent
HK46	Lim et al., 1999	Very virulent

HK46-NT	Lim et al., 1999	Not determined
---------	------------------	----------------

Table 8: Amino acid sequence of the hypervariable region of VP2 of different IBDV isolates. The sequence of the hydrophilic regions (underlined) and the hydrophobic region of very virulent isolate D6948 (amino acid 214 to 328) is used as parental isolate for alignment of the other sequences. Identical amino acid are represented by a dash.

D6948	214	ADDIQFSQYQAGGVITLFSANIDATSLSIGGELVFQTSVOGLILGNTYLIQFDGTAVITRAVAADNGLTAGTQNLMPFNIVIPTSIEITQPIYSIKLEIVTSKSGGQAQDQMSWS328
rD6948		-----
UK661		-----
5123		-----
96-B4		-----
96-C4		-----
96-C5		-----
96-C6		-----
97-B3		-----
97-B4		-----
97-B5		-----
97-B6		-----
Zoontjes		-----
Hungary		-----
OKYM		-----
OKYMT		-----
TKSM		-----
TKSMT		-----
HK46		-----
HK46-NT		-----

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Annex to the application documents - subsequently filed sequences listing

[0114]

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 35 40 45
 Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Ala Val Ile Thr
 50 55 60
 Arg Ala Val Ala Ala Asp Asn Gly Leu Thr Ala Gly Thr Asp Asn Leu
 65 70 75 80
 Met Pro Phe Asn Ile Val Ile Pro Thr Ser Glu Ile Thr Gln Pro Ile
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 100 105 110
 Gly Asp Gln Met Ser Trp Ser
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 35 40 45
 Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Thr Val Ile Thr
 50 55 60
 Arg Ala Val Ala Ala Asn Asn Gly Leu Thr Thr Gly Thr Asp Asn Leu
 65 70 75 80
 Leu Pro Phe Asn Ile Val Ile Pro Thr Asn Glu Ile Thr Gln Pro Ile
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Thr Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln Ala
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Gly Asp Gln Met Ser Trp Ser
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Ile Gly Gly Glu Leu Val Phe Asn Thr Ser Val Gln Gly Leu Ala Leu
 35 40 45

Asn Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Thr Val Ile Thr
 50 55 60

Arg Ala Val Ala Ser Asp Asn Gly Leu Thr Thr Gly Ile Asp Asn Leu
 65 70 75 80

Met Pro Phe Asn Ile Val Ile Pro Thr Asn Glu Ile Thr Gln Pro Ile
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 35 40 45

Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Ala Val Ile Thr
 50 55 60
 5 Arg Ala Val Ala Ala Tyr Asn Gly Leu Thr Thr Gly Thr Asp Asn Leu
 65 70 75 80
 Leu Pro Phe Asn Ile Val Ile Pro Thr Asn Glu Ile Thr Gln Pro Ile
 85 90 95
 10 Thr Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln Ala
 100 105 110
 Gly Asp Gln Met Ser Trp Ser
 115

15

<210> 35
 <211> 119
 <212> PRT
 <213> Infectious bursal disease virus

20

<220>
 <221> DOMAIN
 <222> (1)..(119)
 <223> /note="Amino acid sequence of the hypervariable
 region of VP2 of a IBDV isolate"

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<400> 35
 Ala Ala Asp Asp Tyr Gln Phe Ser Ser Gln Tyr Gln Ala Gly Gly Val
 1 5 10 15
 30 Thr Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu Ser
 20 25 30
 Ile Gly Gly Glu Leu Val Phe Gln Thr Ser Val Gln Gly Leu Ile Leu
 35 40 45
 35 Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Ala Val Ile Thr
 50 55 60
 Arg Ala Val Ala Ala Asn Asn Gly Leu Thr Ala Gly Thr Asp Asn Leu
 65 70 75 80
 40 Met Pro Phe Asn Ile Val Ile Pro Thr Ser Glu Ile Thr Gln Pro Ile
 85 90 95
 Thr Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln Ala
 100 105 110
 Gly Asp Gln Met Ser Trp Ser
 115

45

<210> 36
 <211> 119
 <212> PRT
 <213> Infectious bursal disease virus

50

<220>
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 <222> (1)..(119)
 <223> /note="Amino acid sequence of the hypervariable
 region of VP2 of a IBDV isolate"

55

<400> 36

Ala Ala Asp Asp Tyr Gln Phe Ser Ser Gln Tyr Gln Ala Gly Gly Val
 1 5 10 15

Thr Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu Ser
 20 25 30

Ile Gly Gly Glu Leu Val Phe Gln Thr Ser Val Gln Gly Leu Ile Leu
 35 40 45

Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Ala Val Ile Thr
 50 55 60

Arg Ala Val Ala Ala Asp Asn Gly Leu Thr Ala Gly Thr Asp Asn Leu
 65 70 75 80

Met Pro Phe Asn Ile Val Ile Pro Thr Ser Glu Thr Thr Gln Pro Ile
 85 90 95

Thr Ser Ile Lys Leu Val Ile Val Thr Ser Lys Ser Gly Gly Gln Ala
 100 105 110

Gly Asp Gln Met Ser Trp Ser
 115

<210> 37

<211> 119

<212> PRT

<213> Infectious bursal disease virus

<220>

<221> DOMAIN

<222> (1)...(119)

<223> /note="Amino acid sequence of the hypervariable region of VP2 of a IBDV isolate"

<400> 37

Ala Ala Asp Asp Tyr Gln Phe Ser Ser Gln Tyr Gln Ala Gly Gly Val
 1 5 10 15

Thr Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu Ser
 20 25 30

Ile Gly Gly Glu Leu Val Phe Gln Thr Ser Val Gln Gly Leu Thr Leu
 35 40 45

Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Ala Val Ile Thr
 50 55 60

Arg Ala Val Ala Ala Asn Asn Gly Leu Thr Thr Gly Thr Asp Asn Leu
 65 70 75 80

Met Pro Phe Asn Ile Val Ile Pro Thr Ser Glu Ile Thr Gln Pro Ile
 85 90 95

Thr Ser Ile Lys Leu Glu Ile Val Thr Phe Lys Ser Gly Gly Gln Ala
 100 105 110

Gly Asp Gln Met Ser Trp Ser
 115

<210> 38
 <211> 119
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 <213> Infectious bursal disease virus

<220>
 <221> DOMAIN
 <222> (1)..(119)
 <223> /note="Amino acid sequence of the hypervariable
 region of VP2 of a IBDV isolate"

<400> 38
 Ala Ala Asp Asp Tyr Gln Phe Ser Ser Gln Tyr Gln Ala Gly Gly Val
 1 5 10 15
 Thr Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu Ser
 20 25 30
 Ile Gly Gly Glu Leu Val Phe His Thr Ser Val Gln Gly Leu Ile Leu
 35 40 45
 Asp Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Ala Val Thr Thr
 50 55 60
 Arg Ala Val Ala Ala Asn Asn Gly Leu Thr Thr Gly Thr Asp Asn Leu
 65 70 75 80
 Met Pro Phe Asn Ile Val Ile Pro Thr Ser Glu Ile Thr Gln Pro Ile
 85 90 95
 Thr Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln Ala
 100 105 110
 Gly Asp Gln Met Ser Trp Ser
 115

<210> 39
 <211> 119
 <212> PRT
 <213> Infectious bursal disease virus

<220>
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 <222> (1)..(119)
 <223> /note="Amino acid sequence of the hypervariable
 region of VP2 of a IBDV isolate"

<400> 39
 Ala Ala Asp Asp Tyr Gln Phe Ser Ser Gln Tyr Gln Ala Gly Gly Val
 1 5 10 15
 Thr Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu Ser
 20 25 30
 Ile Gly Gly Glu Leu Val Phe Gln Thr Ser Val Gln Gly Leu Ile Leu
 35 40 45
 Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Ala Val Ile Thr
 50 55 60
 Arg Ala Val Ala Ala Asn Asn Gly Leu Thr Thr Gly Thr Asp Asn Leu
 65 70 75 80
 Met Pro Phe Asn Ile Val Ile Pro Thr Ser Glu Ile Thr Gln Pro Ile

85

90

95

Thr Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln Ala
 100 105 110

Gly Asp Gln Met Ser Trp Ser
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<210> 40
 <211> 3260
 <212> DNA
 <213> Infectious bursal disease virus

<220>
 <221> misc_feature
 <222> (1)..(3260)
 <223> /note="cDNA sequence of IBDV A-segment"

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 gatcgacgcg atgacraacc tgcaagatca aaccacaacag attgttccgt tcatacggag 180
 ccttctgatg ccaacaaccg gaccggcgtc cattccggac gacaccctrg agaagcacac 240
 tctcaggta gagacctga cctacaattt gactgtgggg gacacagggt cagggtctaat 300
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 ctactgcagg ctagtgaatc ggagtctcac agtgaggtca agcactctc ctgggtggcg 480
 ttatgcacta aayggcacca taaacggcgt gaccttccaa ggaagcctga gtgaactgac 540
 agatgttagc tacaatgggt tgatgtctgc aacagccaac atcaacgaca aaatygggaa 600
 cgtcctagta ggggaagggg tmaccgtcct cagcttacc acatcatatg atcttgggta 660
 tgtgagcty ggtgacccca ttccgcwat agggctygac ccaaaaatgg tagcmactg 720
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 aacmarcgag ataaccacgc caatcacatc catcaaatg gagatagtga cctccaaaag 1080
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1860
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 1980
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 2580
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 2640
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 2760
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 2820
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 3000
 maaccargar cagatgaarg ayctgctcyt gactgcatg gagatgaagc atcgcaatcc
 3060
 caggcgggct cyaccaaagc cmaagccaaa acccaatgct ccawcacaga gacccccctgg
 3120
 wcggctgggc cgctggatca ggrcbgtctc tgaygaggac ytkgagtga gycwctggga
 3180
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 3240
 ggatccgttc gcgggtcccc
 3260

 <210> 41
 <211> 3260
 <212> DNA
 <213> Infectious bursal disease virus

 <220>
 <221> misc_feature
 <222> (1)..(3260).
 <223> /note="cDNA sequence of IBV A-segment"

 <400> 41
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 gatcgacg atgacaaacc tgcaagatca aacccaacag attgttccgt tcatacggag 180
 cctctgatg ccaacaaccg gaccggcgtc cattccggac gacaccctgg agaagcacac 240

5 tctcaggtca gagacctcga cctacaattt gactgtgggg gacacagggt cagggctaata 300
 tgtctttttc cctggattcc ctggtcfaat tgtgggtgct cactacacac tgcagagcaa 360
 tgggaactac aagttcgatc agatgtcctt gactgcccag aacctaccgg ccagttacaa 420
 ctactgcagg ctagtgaatc ggagttctac agtgaggtca agcacacttc ctgggtggcgt 480
 ttatgcacta aacggcacca taaacgccgt gaccttccaa ggaagcctga gtgaactgac 540
 agatgttagc tacaatgggt tgatgtctgc aacagccaac atcaacgaca aaattgggaa 600
 cgtcctagta ggggaagggg tcaccgtcct cagcttacc acatcatatg atcttgggta 660
 tgtgaggctt ggtgaccca ttcccgaat agggcttgac ccaaaaatgg tagccacatg 720
 10 tgacagcagt gacagggcca gactctacac cataactgca gccgatgatt accaattctc 780
 atcacagtac caaccagggt gggtacaat cacactgttc tcagccaaca ttgatgccat 840
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 1080
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 1200
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 1260
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 1320
 cacaataatg atactgagt agagggaccg tcttggcatc aagaccgtct ggccaacaag
 1380
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 1440
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 1560
 30 agactacctg ctgggcatg aggcacaggc tgcttcagga actgctcag ccgctcagg
 1620
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 35 ttcacctggg gtactccgag gtgcacacaa cctcgactgc gtgttaagag aggggtgccac
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 1860
 caaatgttt gctgtcattg aaggcgtgcg agaagacctc caacctcct ctcaaagagg
 1920
 40 atccttcata cgaactctct ctggacacag agtctatgga tatgctccag atgggggtact
 1980
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 2040
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 2100
 agcttacatg gatgtgtttc gacccaaagt cccaatccat gtggctatga cgggagccct
 2160
 45 caatgcttgt ggcgagattg agaaagtaag ctttagaagc accaagctcg ccaactgcaca
 2220
 ccgacttggc cttaagttgg ctggtcccgg agcattcgat gtaaacaccg ggcccaactg
 2280
 ggcaacgttc atcaaacgtt tccctcacia tccacgcgac tgggacaggc tcccctacct
 2340
 50 caacctacca tacctccac ccaatgcagg acgccagtac caccttgcca tggctgchtc
 2400
 agagttcaaa gagacccccg aactcgagag tgccgtcaga gcaatggaag cagcagccaa
 2460
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 2520
 55 tgtgactgac atggccaact tcgcactcag cgacccgaac gcccatcgga tgcgaaattt
 2580

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 2700
 gatctcaaag aagatggaga ccatgggcat ctactttgca acaccagaat gggtagcact
 2760
 caatgggcac cgagggccaa gccccggcca gctaaagtac tggcagaaca cagcagaaat
 2820
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 3120
 tcggctgggc cgctggatca ggaccgtctc tgatgaggac cttgagtga gtacctggga
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 3240
 ggatccgttc gcgggtcccc
 3260

 <210> 42
 <211> 3260
 <212> DNA
 <213> Infectious bursal disease virus

 <220>
 <221> misc_feature
 <222> (1)..(3260)
 <223> /note="cDNA sequence of IBV A-segment"

 <400> 42
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 gatcgacgag atgacgaacc tgcaagatca aaccaacag attgttccgt tcatacgag 180
 ccttctgatg ccaacaaccg gaccggcgct cattccggac gacaccctag agaagcacac 240
 tctcagggtca gagacctcga cctacaattt gactgtgggg gacacagggc cagggctaata 300
 tgtctttttc cctgggtttc ctgggtcaat tgtgggtgct cactacacac tgcagagcaa 360
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 ttatgcacta aatggcacca taaacgccgt gaccttccaa ggaagcctga gtgaactgac 540
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 1080
 tgggtggtcag gcgggggatc agatgtcatg gtcagcaagt gggagcctag cagtgcagat
 1140
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 1200
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 1260
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 1320
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1380
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 1440
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 5 1500
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 1560
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 1620
 aaaagcaaga gctgcctcag gccgcataag gcagctaact ctgcgcgcgc acaaggggta
 10 1680
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 15 1860
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 20 2040
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 2100
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 30 2400
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 2460
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 2640
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 2700
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 40 2760
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 2820
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 2880
 agaagaacaa atcctaaggg cagctacgtc gatctacggg gctccaggac aggcagaggc
 45 2940
 accccaagcc ttcatagacg aagtcgcca agtctatgaa atcaaccatg ggcgtggccc
 3000
 caaccaagaa cagatgaaag atctgctcct gactgcgatg gagatgaagc atcgcaatcc
 3060
 caggcggggt ccaccaaagc ccaagccaaa acccaatgct ccaacacaga gacccctgg
 50 3120
 tcggctgggc cgctggatca gggctgtctc tgatgaggac cttgagttag gtacctggga
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 55 3260

5 <210> 43
 <211> 964
 <212> DNA
 <213> Infectious bursal disease virus

 <220>
 <221> misc_feature
 <222> (1)..(964)
 10 <223> /note="cDNA sequence of IBDV A-segment"

<400> 43
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 15 ccagaactcg aagacgctgt gcgtgcaatg gacgctgctg caaacgtcga cccattgttc 180
 cgctcagctc tccaggtctt catgtggttg gaagaaaacg ggattgtaac cgacatggct 240
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<222> (1)..(2827)

<223> /note="cDNA sequence of IBV B-segment"

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 <223> /note="Consensus sequence of IBDV polyprotein, whereby X
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	Met Gly Ile Tyr Phe Ala Thr Pro Glu Trp Val Ala Leu Asn Gly His 865 870 875 880		
25	Arg Gly Pro Ser Pro Gly Gln Leu Lys Tyr Trp Gln Asn Thr Arg Glu 885 890 895		
	Ile Pro Asp Pro Asn Glu Asp Tyr Leu Asp Tyr Val His Ala Glu Lys 900 905 910		
30	Ser Arg Leu Ala Ser Glu Glu Gln Ile Leu Arg Ala Ala Thr Ser Ile 915 920 925		
	Tyr Gly Ala Pro Gly Gln Ala Glu Pro Pro Gln Ala Phe Ile Asp Glu 930 935 940		
35	Val Ala Lys Val Tyr Glu Ile Asn His Gly Arg Gly Pro Asn Gln Glu 945 950 955 960		
	Gln Met Lys Asp Leu Leu Leu Thr Ala Met Glu Met Lys His Arg Asn 965 970 975		
40	Pro Arg Arg Ala Pro Pro Lys Pro Lys Pro Lys Pro Asn Ala Pro Thr 980 985 990		
	Gln Arg Pro Pro Gly Arg Leu Gly Arg Trp Ile Arg Thr Val Ser Asp 995 1000 1005		
45	Glu Asp Leu Glu 1010		
50	<210> 48 <211> 1012 <212> PRT <213> Infectious bursal disease virus		
55	<220> <221> DOMAIN <222> (1)..(1012) <223> /note="Sequence of IBDV polyprotein CEF94-PP" <400> 48		

	Met Thr Asn Leu Gln Asp Gln Thr Gln Gln Ile Val Pro Phe Ile Arg	1	5	10	15
5	Ser Leu Leu Met Pro Thr Thr Gly Pro Ala Ser Ile Pro Asp Asp Thr	20	25	30	
	Leu Glu Lys His Thr Leu Arg Ser Glu Thr Ser Thr Tyr Asn Leu Thr	35	40	45	
10	Val Gly Asp Thr Gly Ser Gly Leu Ile Val Phe Phe Pro Gly Phe Pro	50	55	60	
	Gly Ser Ile Val Gly Ala His Tyr Thr Leu Gln Ser Asn Gly Asn Tyr	65	70	75	80
15	Lys Phe Asp Gln Met Leu Leu Thr Ala Gln Asn Leu Pro Ala Ser Tyr	85	90	95	
	Asn Tyr Cys Arg Leu Val Ser Arg Ser Leu Thr Val Arg Ser Ser Thr	100	105	110	
20	Leu Pro Gly Gly Val Tyr Ala Leu Asn Gly Thr Ile Asn Ala Val Thr	115	120	125	
	Phe Gln Gly Ser Leu Ser Glu Leu Thr Asp Val Ser Tyr Asn Gly Leu	130	135	140	
25	Met Ser Ala Thr Ala Asn Ile Asn Asp Lys Ile Gly Asn Val Leu Val	145	150	155	160
	Gly Glu Gly Val Thr Val Leu Ser Leu Pro Thr Ser Tyr Asp Leu Gly	165	170	175	
30	Tyr Val Arg Leu Gly Asp Pro Ile Pro Ala Ile Gly Leu Asp Pro Lys	180	185	190	
	Met Val Ala Thr Cys Asp Ser Ser Asp Arg Pro Arg Val Tyr Thr Ile	195	200	205	
35	Thr Ala Ala Asp Asp Tyr Gln Phe Ser Ser Gln Tyr Gln Pro Gly Gly	210	215	220	
	Val Thr Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu	225	230	235	240
40	Ser Val Gly Gly Glu Leu Val Phe Gln Thr Ser Val His Gly Leu Val	245	250	255	
	Leu Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Ala Val Ile	260	265	270	
45	Thr Arg Ala Val Ala Ala Asn Asn Gly Leu Thr Thr Gly Thr Asp Asn	275	280	285	
	Leu Leu Pro Phe Asn Leu Val Ile Pro Thr Asn Glu Ile Thr Gln Pro	290	295	300	
50	Ile Thr Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln	305	310	315	320
	Ala Gly Asp Gln Met Ser Trp Ser Ala Arg Gly Ser Leu Ala Val Thr	325	330	335	
55	Ile His Gly Gly Asn Tyr Pro Gly Ala Leu Arg Pro Val Thr Leu Val	340	345	350	

	Ala Tyr Glu Arg Val Ala Thr Gly Ser Val Val Thr Val Ala Gly Val	355	360	365
5	Ser Asn Phe Glu Leu Ile Pro Asn Pro Glu Leu Ala Lys Asn Leu Val	370	375	380
	Thr Glu Tyr Gly Arg Phe Asp Pro Gly Ala Met Asn Tyr Thr Lys Leu	385	390	395
10	Ile Leu Ser Glu Arg Asp Arg Leu Gly Ile Lys Thr Val Trp Pro Thr	405	410	415
	Arg Glu Tyr Thr Asp Phe Arg Glu Tyr Phe Met Glu Val Ala Asp Leu	420	425	430
15	Asn Ser Pro Leu Lys Ile Ala Gly Ala Phe Gly Phe Lys Asp Ile Ile	435	440	445
	Arg Ala Ile Arg Arg Ile Ala Val Pro Val Val Ser Thr Leu Phe Pro	450	455	460
20	Pro Ala Ala Pro Leu Ala His Ala Ile Gly Glu Gly Val Asp Tyr Leu	465	470	475
	Leu Gly Asp Glu Ala Gln Ala Ala Ser Gly Thr Ala Arg Ala Ala Ser	485	490	495
25	Gly Lys Ala Arg Ala Ala Ser Gly Arg Ile Arg Gln Leu Thr Leu Ala	500	505	510
	Ala Asp Lys Gly Tyr Glu Val Val Ala Asn Leu Phe Gln Val Pro Gln	515	520	525
30	Asn Pro Val Val Asp Gly Ile Leu Ala Ser Pro Gly Val Leu Arg Gly	530	535	540
	Ala His Asn Leu Asp Cys Val Leu Arg Glu Gly Ala Thr Leu Phe Pro	545	550	555
35	Val Val Ile Thr Thr Val Glu Asp Ala Met Thr Pro Lys Ala Leu Asn	565	570	575
	Ser Lys Met Phe Ala Val Ile Glu Gly Val Arg Glu Asp Leu Gln Pro	580	585	590
40	Pro Ser Gln Arg Gly Ser Phe Ile Arg Thr Leu Ser Gly His Arg Val	595	600	605
	Tyr Gly Tyr Ala Pro Asp Gly Val Leu Pro Leu Glu Thr Gly Arg Asp	610	615	620
45	Tyr Thr Val Val Pro Ile Asp Asp Val Trp Asp Asp Ser Ile Met Leu	625	630	635
	Ser Lys Asp Pro Ile Pro Pro Ile Val Gly Asn Ser Gly Asn Leu Ala	645	650	655
50	Ile Ala Tyr Met Asp Val Phe Arg Pro Lys Val Pro Ile His Val Ala	660	665	670
	Met Thr Gly Ala Leu Asn Ala Cys Gly Glu Ile Glu Lys Val Ser Phe	675	680	685
55	Arg Ser Thr Lys Leu Ala Thr Ala His Arg Leu Gly Leu Lys Leu Ala	690	695	700

Gly Pro Gly Ala Phe Asp Val Asn Thr Gly Pro Asn Trp Ala Thr Phe
 705 710 715 720
 5 Ile Lys Arg Phe Pro His Asn Pro Arg Asp Trp Asp Arg Leu Pro Tyr
 725 730 735
 Leu Asn Leu Pro Tyr Leu Pro Pro Asn Ala Gly Arg Gln Tyr His Leu
 740 745 750
 10 Ala Met Ala Ala Ser Glu Phe Lys Glu Thr Pro Glu Leu Glu Ser Ala
 755 760 765
 Val Arg Ala Met Glu Ala Ala Ala Asn Val Asp Pro Leu Phe Gln Ser
 770 775 780
 15 Ala Leu Ser Val Phe Met Trp Leu Glu Glu Asn Gly Ile Val Thr Asp
 785 790 795 800
 Met Ala Asn Phe Ala Leu Ser Asp Pro Asn Ala His Arg Met Arg Asn
 805 810 815
 20 Phe Leu Ala Asn Ala Pro Gln Ala Gly Ser Lys Ser Gln Arg Ala Lys
 820 825 830
 Tyr Gly Thr Ala Gly Tyr Gly Val Glu Ala Arg Gly Pro Thr Pro Glu
 835 840 845
 25 Glu Ala Gln Arg Glu Lys Asp Thr Arg Ile Ser Lys Lys Met Glu Thr
 850 855 860
 Met Gly Ile Tyr Phe Ala Thr Pro Glu Trp Val Ala Leu Asn Gly His
 865 870 875 880
 30 Arg Gly Pro Ser Pro Gly Gln Leu Lys Tyr Trp Gln Asn Thr Arg Glu
 885 890 895
 Ile Pro Asp Pro Asn Glu Asp Tyr Leu Asp Tyr Val His Ala Glu Lys
 900 905 910
 35 Ser Arg Leu Ala Ser Glu Glu Gln Ile Leu Arg Ala Ala Thr Ser Ile
 915 920 925
 Tyr Gly Ala Pro Gly Gln Ala Glu Pro Pro Gln Ala Phe Ile Asp Glu
 930 935 940
 40 Val Ala Lys Val Tyr Glu Ile Asn His Gly Arg Gly Pro Asn Gln Glu
 945 950 955 960
 Gln Met Lys Asp Leu Leu Leu Thr Ala Met Glu Met Lys His Arg Asn
 965 970 975
 45 Pro Arg Arg Ala Pro Pro Lys Pro Lys Pro Lys Pro Asn Ala Pro Thr
 980 985 990
 Gln Arg Pro Pro Gly Arg Leu Gly Arg Trp Ile Arg Thr Val Ser Asp
 995 1000 1005
 50 Glu Asp Leu Glu
 1010

<210> 49

<211> 1012

<212> PRT

<213> Infectious bursal disease virus

<220>
 <221> DOMAIN
 <222> (1)..(1012)
 <223> /note="Sequence of IBDV polyprotein D6948-PP"

5

<400> 49

Met Thr Asn Leu Gln Asp Gln Thr Gln Gln Ile Val Pro Phe Ile Arg
 1 5 10 15

10

Ser Leu Leu Met Pro Thr Thr Gly Pro Ala Ser Ile Pro Asp Asp Thr
 20 25 30

Leu Glu Lys His Thr Leu Arg Ser Glu Thr Ser Thr Tyr Asn Leu Thr
 35 40 45

15

Val Gly Asp Thr Gly Ser Gly Leu Ile Val Phe Phe Pro Gly Phe Pro
 50 55 60

Gly Ser Ile Val Gly Ala His Tyr Thr Leu Gln Ser Asn Gly Asn Tyr
 65 70 75 80

20

Lys Phe Asp Gln Met Leu Leu Thr Ala Gln Asn Leu Pro Ala Ser Tyr
 85 90 95

Asn Tyr Cys Arg Leu Val Ser Arg Ser Leu Thr Val Arg Ser Ser Thr
 100 105 110

25

Leu Pro Gly Gly Val Tyr Ala Leu Asn Gly Thr Ile Asn Ala Val Thr
 115 120 125

Phe Gln Gly Ser Leu Ser Glu Leu Thr Asp Val Ser Tyr Asn Gly Leu
 130 135 140

30

Met Ser Ala Thr Ala Asn Ile Asn Asp Lys Ile Gly Asn Val Leu Val
 145 150 155 160

Gly Glu Gly Val Thr Val Leu Ser Leu Pro Thr Ser Tyr Asp Leu Gly
 165 170 175

35

Tyr Val Arg Leu Gly Asp Pro Ile Pro Ala Ile Gly Leu Asp Pro Lys
 180 185 190

Met Val Ala Thr Cys Asp Ser Ser Asp Arg Pro Arg Val Tyr Thr Ile
 195 200 205

40

Thr Ala Ala Asp Asp Tyr Gln Phe Ser Ser Gln Tyr Gln Ala Gly Gly
 210 215 220

Val Thr Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu
 225 230 235 240

45

Ser Ile Gly Gly Glu Leu Val Phe Gln Thr Ser Val Gln Gly Leu Ile
 245 250 255

Leu Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Ala Val Ile
 260 265 270

50

Thr Arg Ala Val Ala Ala Asp Asn Gly Leu Thr Ala Gly Thr Asp Asn
 275 280 285

Leu Met Pro Phe Asn Ile Val Ile Pro Thr Ser Glu Ile Thr Gln Pro
 290 295 300

55

Ile Thr Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln
 305 310 315 320

Ala Gly Asp Gln Met Ser Trp Ser Ala Ser Gly Ser Leu Ala Val Thr
325 330 335

5 Ile His Gly Gly Asn Tyr Pro Gly Ala Leu Arg Pro Val Thr Leu Val
340 345 350

Ala Tyr Glu Arg Val Ala Thr Gly Ser Val Val Thr Val Ala Gly Val
355 360 365

10 Ser Asn Phe Glu Leu Ile Pro Asn Pro Glu Leu Ala Lys Asn Leu Val
370 375 380

Thr Glu Tyr Gly Arg Phe Asp Pro Gly Ala Met Asn Tyr Thr Lys Leu
385 390 395 400

15 Ile Leu Ser Glu Arg Asp Arg Leu Gly Ile Lys Thr Val Trp Pro Thr
405 410 415

Arg Glu Tyr Thr Asp Phe Arg Glu Tyr Phe Met Glu Val Ala Asp Leu
420 425 430

20 Asn Ser Pro Leu Lys Ile Ala Gly Ala Phe Gly Phe Lys Asp Ile Ile
435 440 445

Arg Ala Leu Arg Arg Ile Ala Val Pro Val Val Ser Thr Leu Phe Pro
450 455 460

25 Pro Ala Ala Pro Leu Ala His Ala Ile Gly Glu Gly Val Asp Tyr Leu
465 470 475 480

Leu Gly Asp Glu Ala Gln Ala Ala Ser Gly Thr Ala Arg Ala Ala Ser
485 490 495

30 Gly Lys Ala Arg Ala Ala Ser Gly Arg Ile Arg Gln Leu Thr Leu Ala
500 505 510

Ala Asp Lys Gly Tyr Glu Val Val Ala Asn Leu Phe Gln Val Pro Gln
515 520 525

35 Asn Pro Val Val Asp Gly Ile Leu Ala Ser Pro Gly Ile Leu Arg Gly
530 535 540

Ala His Asn Leu Asp Cys Val Leu Arg Glu Gly Ala Thr Leu Phe Pro
545 550 555 560

40 Val Val Ile Thr Thr Val Glu Asp Ala Met Thr Pro Lys Ala Leu Asn
565 570 575

Ser Lys Met Phe Ala Val Ile Glu Gly Val Arg Glu Asp Leu Gln Pro
580 585 590

45 Pro Ser Gln Arg Gly Ser Phe Ile Arg Thr Leu Ser Gly His Arg Val
595 600 605

Tyr Gly Tyr Ala Pro Asp Gly Val Leu Pro Leu Glu Thr Gly Arg Asp
610 615 620

50 Tyr Thr Val Val Pro Ile Asp Asp Val Trp Asp Asp Ser Ile Met Leu
625 630 635 640

Ser Lys Asp Pro Ile Pro Pro Ile Val Gly Asn Ser Gly Asn Leu Ala
645 650 655

55 Ile Ala Tyr Met Asp Val Phe Arg Pro Lys Val Pro Ile His Val Ala
660 665 670

	Met	Thr	Gly	Ala	Leu	Asn	Ala	Tyr	Gly	Glu	Ile	Glu	Asn	Val	Ser	Phe	
			675					680					685				
5	Arg	Ser	Thr	Lys	Leu	Ala	Thr	Ala	His	Arg	Leu	Gly	Leu	Lys	Leu	Ala	
		690					695					700					
	Gly	Pro	Gly	Ala	Phe	Asp	Val	Asn	Thr	Gly	Ser	Asn	Trp	Ala	Thr	Phe	
	705					710					715					720	
10	Ile	Lys	Arg	Phe	Pro	His	Asn	Pro	Arg	Asp	Trp	Asp	Arg	Leu	Pro	Tyr	
					725					730					735		
	Leu	Asn	Leu	Pro	Tyr	Leu	Pro	Pro	Asn	Ala	Gly	Arg	Gln	Tyr	Asp	Leu	
				740					745					750			
15	Ala	Met	Ala	Ala	Ser	Glu	Phe	Lys	Glu	Thr	Pro	Glu	Leu	Glu	Ser	Ala	
		755						760					765				
	Val	Arg	Ala	Met	Glu	Ala	Ala	Ala	Asn	Val	Asp	Pro	Leu	Phe	Gln	Ser	
	770					775						780					
20	Ala	Leu	Ser	Val	Phe	Met	Trp	Leu	Glu	Glu	Asn	Gly	Ile	Val	Thr	Asp	
	785					790						795				800	
	Met	Ala	Asn	Phe	Ala	Leu	Ser	Asp	Pro	Asn	Ala	His	Arg	Met	Arg	Asn	
				805						810					815		
25	Phe	Leu	Ala	Asn	Ala	Pro	Gln	Ala	Gly	Ser	Lys	Ser	Gln	Arg	Ala	Lys	
				820					825					830			
	Tyr	Gly	Thr	Ala	Gly	Tyr	Gly	Val	Glu	Ala	Arg	Gly	Pro	Thr	Pro	Glu	
		835						840					845				
30	Glu	Ala	Gln	Arg	Glu	Lys	Asp	Thr	Arg	Ile	Ser	Lys	Lys	Met	Glu	Thr	
	850						855					860					
	Met	Gly	Ile	Tyr	Phe	Ala	Thr	Pro	Glu	Trp	Val	Ala	Leu	Asn	Gly	His	
	865					870					875					880	
35	Arg	Gly	Pro	Ser	Pro	Gly	Gln	Leu	Lys	Tyr	Trp	Gln	Asn	Thr	Arg	Glu	
					885					890					895		
	Ile	Pro	Asp	Pro	Asn	Glu	Asp	Tyr	Leu	Asp	Tyr	Val	His	Ala	Glu	Lys	
				900					905					910			
40	Ser	Arg	Leu	Ala	Ser	Glu	Glu	Gln	Ile	Leu	Arg	Ala	Ala	Thr	Ser	Ile	
		915						920					925				
	Tyr	Gly	Ala	Pro	Gly	Gln	Ala	Glu	Pro	Pro	Gln	Ala	Phe	Ile	Asp	Glu	
	930					935						940					
45	Val	Ala	Lys	Val	Tyr	Glu	Ile	Asn	His	Gly	Arg	Gly	Pro	Asn	Gln	Glu	
	945					950					955					960	
	Gln	Met	Lys	Asp	Leu	Leu	Leu	Thr	Ala	Met	Glu	Met	Lys	His	Arg	Asn	
				965						970					975		
50	Pro	Arg	Arg	Ala	Pro	Pro	Lys	Pro	Lys	Pro	Lys	Pro	Asn	Ala	Pro	Thr	
				980					985					990			
	Gln	Arg	Pro	Pro	Gly	Arg	Leu	Gly	Arg	Trp	Ile	Arg	Ala	Val	Ser	Asp	
		995					1000						1005				
55	Glu	Asp	Leu	Glu													
	1010																

<210> 50
 <211> 290
 <212> PRT
 <213> Infectious bursal disease virus

<220>
 <221> DOMAIN
 <222> (1)..(290)
 <223> /note="Sequence of IBDV polyprotein TY89-PP"

<400> 50

Arg Phe Pro His Asn Pro Arg Asp Trp Asp Arg Leu Pro Tyr Leu Asn
 1 5 10 15

Leu Pro Tyr Leu Pro Pro Thr Ala Gly Arg Gln Phe His Leu Ala Leu
 20 25 30

Ala Ala Ser Glu Phe Lys Glu Thr Pro Glu Leu Glu Asp Ala Val Arg
 35 40 45

Ala Met Asp Ala Ala Ala Asn Val Asp Pro Leu Phe Arg Ser Ala Leu
 50 55 60

Gln Val Phe Met Trp Leu Glu Glu Asn Gly Ile Val Thr Asp Met Ala
 65 70 75 80

Asn Phe Ala Leu Ser Asp Pro Asn Ala His Arg Met Lys Asn Phe Leu
 85 90 95

Ala Asn Ala Pro Gln Ala Gly Ser Lys Ser Gln Arg Ala Lys Tyr Gly
 100 105 110

Thr Ala Gly Tyr Gly Val Glu Ala Arg Gly Pro Thr Pro Glu Glu Ala
 115 120 125

Gln Arg Glu Lys Asp Thr Arg Ile Ser Lys Lys Met Glu Thr Met Gly
 130 135 140

Ile Tyr Phe Ala Thr Pro Glu Trp Val Ala Leu Asn Gly His Arg Gly
 145 150 155 160

Pro Ser Pro Gly Gln Leu Lys Tyr Trp Gln Asn Thr Arg Glu Ile Pro
 165 170 175

Glu Pro Asn Glu Asp Tyr Pro Asp Tyr Val His Ala Glu Lys Ser Arg
 180 185 190

Leu Ala Ser Glu Glu Gln Val Leu Arg Ala Ala Thr Ser Ile Tyr Gly
 195 200 205

Ala Pro Gly Gln Ala Glu Pro Pro Gln Ala Phe Ile Asp Glu Val Ala
 210 215 220

Arg Val Tyr Glu Ile Asn His Gly Arg Gly Pro Asn Gln Glu Gln Met
 225 230 235 240

Lys Asp Leu Leu Leu Thr Ala Met Glu Met Lys His Arg Asn Pro Arg
 245 250 255

Arg Ala Pro Pro Lys Pro Lys Pro Lys Pro Asn Ala Pro Ser Gln Arg
 260 265 270

Pro Pro Gly Arg Leu Gly Arg Trp Ile Arg Thr Val Ser Asp Glu Asp

275

280

285

Leu Glu
290

5

<210> 51

<211> 881

<212> PRT

10

<213> Infectious bursal disease virus

<220>

<221> DOMAIN

<222> (1)..(881)

15

<223> /note="Consensus sequence of IBV VP1, whereby X stands for any amino acid"

<400> 51

Met Ser Asp Xaa Phe Asn Ser Pro Gln Ala Arg Ser Xaa Ile Ser Ala
1 5 10 15

20

Ala Phe Gly Ile Lys Pro Thr Ala Gly Gln Asp Val Glu Glu Leu Leu
20 25 30

Ile Pro Lys Val Trp Val Pro Pro Glu Asp Pro Leu Ala Ser Pro Ser
35 40 45

25

Arg Leu Ala Lys Phe Leu Arg Glu Asn Gly Tyr Lys Xaa Leu Gln Pro
50 55 60

Arg Ser Leu Pro Glu Asn Glu Glu Tyr Glu Thr Asp Gln Ile Leu Pro
65 70 75 80

30

Asp Leu Ala Trp Met Arg Gln Ile Glu Gly Ala Val Leu Lys Pro Thr
85 90 95

Leu Ser Leu Pro Ile Gly Asp Gln Glu Tyr Phe Pro Lys Tyr Tyr Pro
100 105 110

35

Thr His Arg Pro Ser Lys Glu Lys Pro Asn Ala Tyr Pro Pro Asp Ile
115 120 125

Ala Leu Leu Lys Gln Met Ile Tyr Leu Phe Leu Gln Val Pro Glu Ala
130 135 140

40

Xaa Xaa Xaa Leu Lys Asp Glu Val Thr Leu Leu Thr Gln Asn Ile Arg
145 150 155 160

Asp Lys Ala Tyr Gly Ser Gly Thr Tyr Met Gly Gln Ala Thr Arg Leu
165 170 175

45

Val Ala Met Lys Glu Val Ala Thr Gly Arg Asn Pro Asn Lys Asp Pro
180 185 190

Leu Lys Leu Gly Tyr Thr Phe Glu Ser Ile Ala Gln Leu Leu Asp Ile
195 200 205

50

Thr Leu Pro Val Gly Pro Pro Gly Glu Asp Asp Lys Pro Trp Val Pro
210 215 220

Leu Thr Arg Val Pro Ser Arg Met Leu Val Leu Thr Gly Asp Val Asp
225 230 235 240

55

Gly Xaa Phe Glu Val Glu Asp Tyr Leu Pro Lys Ile Asn Leu Lys Ser
245 250 255

5 Ser Ser Gly Leu Pro Tyr Val Gly Arg Thr Lys Gly Glu Thr Ile Gly
 260 265 270
 Glu Met Ile Ala Ile Ser Asn Gln Phe Leu Arg Glu Leu Ser Xaa Leu
 275 280 285
 10 Leu Lys Gln Gly Ala Gly Thr Lys Gly Ser Asn Lys Lys Lys Leu Leu
 290 295 300
 Ser Met Leu Ser Asp Tyr Trp Tyr Leu Ser Cys Gly Leu Leu Phe Pro
 305 310 315 320
 15 Lys Ala Glu Arg Tyr Asp Lys Ser Thr Trp Leu Thr Lys Thr Arg Asn
 325 330 335
 Ile Trp Ser Ala Pro Ser Pro Thr His Leu Met Ile Ser Met Ile Thr
 340 345 350
 20 Trp Pro Val Met Ser Asn Ser Pro Asn Asn Val Leu Asn Ile Glu Gly
 355 360 365
 Cys Pro Ser Leu Tyr Lys Phe Asn Pro Phe Arg Gly Gly Leu Asn Arg
 370 375 380
 25 Ile Val Glu Trp Ile Xaa Ala Pro Xaa Glu Pro Lys Ala Leu Val Tyr
 385 390 395 400
 Ala Asp Asn Ile Tyr Ile Val His Ser Asn Thr Trp Tyr Ser Ile Asp
 405 410 415
 30 Leu Glu Lys Gly Glu Ala Asn Cys Thr Arg Gln His Met Gln Ala Ala
 420 425 430
 Met Tyr Tyr Ile Leu Thr Arg Gly Trp Ser Asp Asn Gly Asp Pro Met
 435 440 445
 35 Phe Asn Gln Thr Trp Ala Thr Phe Ala Met Asn Ile Ala Pro Ala Leu
 450 455 460
 Val Val Asp Ser Ser Cys Leu Ile Met Asn Leu Gln Ile Lys Thr Tyr
 465 470 475 480
 40 Gly Gln Gly Ser Gly Asn Ala Ala Thr Phe Ile Asn Asn His Leu Leu
 485 490 495
 Ser Thr Leu Val Leu Asp Gln Trp Asn Leu Met Xaa Gln Pro Xaa Pro
 500 505 510
 45 Asp Ser Glu Glu Phe Lys Ser Ile Glu Asp Lys Leu Gly Ile Asn Phe
 515 520 525
 Lys Ile Glu Arg Ser Ile Asp Asp Ile Arg Gly Lys Leu Arg Gln Leu
 530 535 540
 50 Val Xaa Leu Ala Gln Pro Gly Tyr Leu Ser Gly Gly Val Glu Pro Glu
 545 550 555 560
 Gln Xaa Ser Pro Thr Val Glu Leu Asp Leu Leu Gly Trp Ser Ala Thr
 565 570 575
 Tyr Ser Lys Asp Leu Gly Ile Tyr Val Pro Val Leu Asp Lys Glu Arg
 580 585 590
 55 Leu Phe Cys Ser Ala Ala Tyr Pro Lys Gly Val Glu Asn Lys Ser Leu
 595 600 605

Lys Ser Lys Val Gly Ile Glu Gln Ala Tyr Lys Val Val Arg Tyr Glu
 610 615 620
 5 Ala Leu Arg Leu Val Gly Gly Trp Asn Tyr Pro Leu Leu Asn Lys Ala
 625 630 635 640
 Cys Lys Asn Asn Ala Xaa Ala Ala Arg Arg His Leu Glu Ala Lys Gly
 645 650 655
 10 Phe Pro Leu Asp Glu Phe Leu Ala Glu Trp Ser Glu Leu Ser Glu Phe
 660 665 670
 Gly Glu Ala Phe Glu Gly Phe Asn Ile Lys Leu Thr Val Thr Xaa Glu
 675 680 685
 15 Ser Leu Ala Glu Leu Asn Xaa Pro Val Pro Pro Lys Pro Pro Asn Val
 690 695 700
 Asn Arg Pro Val Asn Thr Gly Gly Leu Lys Ala Val Ser Asn Ala Leu
 705 710 715 720
 20 Lys Thr Gly Arg Tyr Arg Asn Glu Ala Gly Leu Ser Gly Leu Val Leu
 725 730 735
 Leu Ala Thr Ala Arg Ser Arg Leu Gln Asp Ala Val Lys Ala Lys Ala
 740 745 750
 25 Glu Ala Glu Lys Leu His Lys Ser Lys Pro Asp Asp Pro Asp Ala Asp
 755 760 765
 Trp Phe Glu Arg Ser Glu Thr Leu Ser Asp Leu Leu Glu Lys Ala Asp
 770 775 780
 30 Ile Ala Ser Lys Val Ala His Ser Ala Leu Val Glu Thr Ser Asp Ala
 785 790 795 800
 Leu Glu Ala Val Gln Ser Thr Ser Val Tyr Thr Pro Lys Tyr Pro Glu
 805 810 815
 35 Val Lys Asn Pro Gln Thr Ala Ser Asn Pro Val Val Gly Leu His Leu
 820 825 830
 Pro Ala Lys Arg Ala Thr Gly Val Gln Ala Ala Leu Leu Gly Ala Gly
 835 840 845
 40 Thr Ser Arg Pro Met Gly Met Glu Ala Pro Thr Arg Ser Lys Asn Ala
 850 855 860
 Val Lys Met Ala Lys Arg Arg Gln Arg Gln Lys Glu Ser Arg Gln Xaa
 865 870 875 880

45 Xaa

50 <210> 52
 <211> 881
 <212> PRT
 <213> Infectious bursal disease virus
 <220>
 <221> DOMAIN
 <222> (1)..(881)
 55 <223> /note="Sequence of IBDV CEF94-VP1"

<400> 52

5 Met Ser Asp Ile Phe Asn Ser Pro Gln Ala Arg Ser Thr Ile Ser Ala
 1 5 10 15
 Ala Phe Gly Ile Lys Pro Thr Ala Gly Gln Asp Val Glu Glu Leu Leu
 20 25 30
 10 Ile Pro Lys Val Trp Val Pro Pro Glu Asp Pro Leu Ala Ser Pro Ser
 35 40 45
 Arg Leu Ala Lys Phe Leu Arg Glu Asn Gly Tyr Lys Val Leu Gln Pro
 50 55 60
 15 Arg Ser Leu Pro Glu Asn Glu Glu Tyr Glu Thr Asp Gln Ile Leu Pro
 65 70 75 80
 Asp Leu Ala Trp Met Arg Gln Ile Glu Gly Ala Val Leu Lys Pro Thr
 85 90 95
 20 Leu Ser Leu Pro Ile Gly Asp Gln Glu Tyr Phe Pro Lys Tyr Tyr Pro
 100 105 110
 Thr His Arg Pro Ser Lys Glu Lys Pro Asn Ala Tyr Pro Pro Asp Ile
 115 120 125
 25 Ala Leu Leu Lys Gln Met Ile Tyr Leu Phe Leu Gln Val Pro Glu Ala
 130 135 140
 Asn Glu Gly Leu Lys Asp Glu Val Thr Leu Leu Thr Gln Asn Ile Arg
 145 150 155 160
 30 Asp Lys Ala Tyr Gly Ser Gly Thr Tyr Met Gly Gln Ala Thr Arg Leu
 165 170 175
 Val Ala Met Lys Glu Val Ala Thr Gly Arg Asn Pro Asn Lys Asp Pro
 180 185 190
 35 Leu Lys Leu Gly Tyr Thr Phe Glu Ser Ile Ala Gln Leu Leu Asp Ile
 195 200 205
 Thr Leu Pro Val Gly Pro Pro Gly Glu Asp Asp Lys Pro Trp Val Pro
 210 215 220
 40 Leu Thr Arg Val Pro Ser Arg Met Leu Val Leu Thr Gly Asp Val Asp
 225 230 235 240
 Gly Asp Phe Glu Val Glu Asp Tyr Leu Pro Lys Ile Asn Leu Lys Ser
 245 250 255
 45 Ser Ser Gly Leu Pro Tyr Val Gly Arg Thr Lys Gly Glu Thr Ile Gly
 260 265 270
 Glu Met Ile Ala Ile Ser Asn Gln Phe Leu Arg Glu Leu Ser Thr Leu
 275 280 285
 50 Leu Lys Gln Gly Ala Gly Thr Lys Gly Ser Asn Lys Lys Lys Leu Leu
 290 295 300
 Ser Met Leu Ser Asp Tyr Trp Tyr Leu Ser Cys Gly Leu Leu Phe Pro
 305 310 315 320
 55 Lys Ala Glu Arg Tyr Asp Lys Ser Thr Trp Leu Thr Lys Thr Arg Asn
 325 330 335
 Ile Trp Ser Ala Pro Ser Pro Thr His Leu Met Ile Ser Met Ile Thr

	340	345	350
5	Trp Pro Val Met Ser Asn Ser 355	Pro Asn Asn Val 360	Leu Asn Ile Glu Gly 365
	Cys Pro Ser Leu Tyr Lys 370	Phe Asn Pro Phe Arg 375	Gly Gly Leu Asn Arg 380
10	Ile Val Glu Trp Ile Leu Ala 385	Pro Glu Glu 390	Pro Lys Ala Leu Val Tyr 395 400
	Ala Asp Asn Ile Tyr Ile Val 405	His Ser Asn Thr 410	Trp Tyr Ser Ile Asp 415
15	Leu Glu Lys Gly Glu Ala Asn 420	Cys Thr Arg Gln His 425	Met Gln Ala Ala 430
	Met Tyr Tyr Ile Leu Thr Arg 435	Gly Trp Ser Asp Asn 440	Gly Asp Pro Met 445
20	Phe Asn Gln Thr Trp Ala Thr 450	Phe Ala Met Asn Ile 455	Ala Pro Ala Leu 460
	Val Val Asp Ser Ser Cys Leu 465	Ile Met Asn Leu Gln 470	Ile Lys Thr Tyr 475 480
25	Gly Gln Gly Ser Gly Asn Ala 485	Ala Thr Phe Ile Asn Asn 490	His Leu Leu 495
	Ser Thr Leu Val Leu Asp Gln 500	Trp Asn Leu Met Arg 505	Gln Pro Arg Pro 510
30	Asp Ser Glu Glu Phe Lys Ser 515	Ile Glu Asp Lys Leu 520	Gly Ile Asn Phe 525
	Lys Ile Glu Arg Ser Ile Asp 530	Asp Ile Arg Gly Lys 535	Leu Arg Gln Leu 540
35	Val Leu Leu Ala Gln Pro 545	Gly Tyr Leu Ser Gly 550	Gly Val Glu Pro Glu 555 560
	Gln Ser Ser Pro Thr Val 565	Glu Leu Asp Leu Gly 570	Trp Ser Ala Thr 575
40	Tyr Ser Lys Asp Leu Gly Ile 580	Tyr Val Pro Val Leu 585	Asp Lys Glu Arg 590
	Leu Phe Cys Ser Ala Ala Tyr 595	Pro Lys Gly Val Glu 600	Asn Lys Ser Leu 605
45	Lys Ser Lys Val Gly Ile Glu 610	Gln Ala Tyr Lys Val 615	Val Arg Tyr Glu 620
	Ala Leu Arg Leu Val Gly 625	Gly Trp Asn Tyr Pro 630	Leu Leu Asn Lys Ala 635 640
50	Cys Lys Asn Asn Ala Gly 645	Ala Ala Arg Arg His 650	Leu Glu Ala Lys Gly 655
	Phe Pro Leu Asp Glu Phe 660	Leu Ala Glu Trp Ser 665	Glu Leu Ser Glu Phe 670
55	Gly Glu Ala Phe Glu Gly 675	Phe Asn Ile Lys Leu 680	Thr Val Thr Ser Glu 685
	Ser Leu Ala Glu Leu Asn 690	Lys Pro Val Pro Pro 695	Lys Pro Pro Asn Val 700

	690	695	700
5	Asn Arg Pro Val Asn Thr Gly Gly Leu Lys Ala Val Ser Asn Ala Leu 705 710 715 720		
	Lys Thr Gly Arg Tyr Arg Asn Glu Ala Gly Leu Ser Gly Leu Val Leu 725 730 735		
10	Leu Ala Thr Ala Arg Ser Arg Leu Gln Asp Ala Val Lys Ala Lys Ala 740 745 750		
	Glu Ala Glu Lys Leu His Lys Ser Lys Pro Asp Asp Pro Asp Ala Asp 755 760 765		
15	Trp Phe Glu Arg Ser Glu Thr Leu Ser Asp Leu Leu Glu Lys Ala Asp 770 775 780		
	Ile Ala Ser Lys Val Ala His Ser Ala Leu Val Glu Thr Ser Asp Ala 785 790 795 800		
20	Leu Glu Ala Val Gln Ser Thr Ser Val Tyr Thr Pro Lys Tyr Pro Glu 805 810 815		
	Val Lys Asn Pro Gln Thr Ala Ser Asn Pro Val Val Gly Leu His Leu 820 825 830		
25	Pro Ala Lys Arg Ala Thr Gly Val Gln Ala Ala Leu Leu Gly Ala Gly 835 840 845		
	Thr Ser Arg Pro Met Gly Met Glu Ala Pro Thr Arg Ser Lys Asn Ala 850 855 860		
30	Val Lys Met Ala Lys Arg Arg Gln Arg Gln Lys Glu Ser Arg Gln Gln 865 870 875 880		
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50	Ile Pro Lys Val Trp Val Pro Pro Glu Asp Pro Leu Ala Ser Pro Ser 35 40 45		
	Arg Leu Ala Lys Phe Leu Arg Glu Asn Gly Tyr Lys Ile Leu Gln Pro 50 55 60		
55	Arg Ser Leu Pro Glu Asn Glu Glu Tyr Glu Thr Asp Gln Ile Leu Pro 65 70 75 80		

5 Asp Leu Ala Trp Met Arg Gln Ile Glu Gly Ala Val Leu Lys Pro Thr
 85 90 95
 Leu Ser Leu Pro Ile Gly Asp Gln Glu Tyr Phe Pro Lys Tyr Tyr Pro
 100 105 110
 10 Thr His Arg Pro Ser Lys Glu Lys Pro Asn Ala Tyr Pro Pro Asp Ile
 115 120 125
 Ala Leu Leu Lys Gln Met Ile Tyr Leu Phe Leu Gln Val Pro Glu Ala
 130 135 140
 15 Thr Asp Asn Leu Lys Asp Glu Val Thr Leu Leu Thr Gln Asn Ile Arg
 145 150 155 160
 Asp Lys Ala Tyr Gly Ser Gly Thr Tyr Met Gly Gln Ala Thr Arg Leu
 165 170 175
 20 Val Ala Met Lys Glu Val Ala Thr Gly Arg Asn Pro Asn Lys Asp Pro
 180 185 190
 Leu Lys Leu Gly Tyr Thr Phe Glu Ser Ile Ala Gln Leu Leu Asp Ile
 195 200 205
 25 Thr Leu Pro Val Gly Pro Pro Gly Glu Asp Asp Lys Pro Trp Val Pro
 210 215 220
 Leu Thr Arg Val Pro Ser Arg Met Leu Val Leu Thr Gly Asp Val Asp
 225 230 235 240
 30 Gly Glu Phe Glu Val Glu Asp Tyr Leu Pro Lys Ile Asn Leu Lys Ser
 245 250 255
 Ser Ser Gly Leu Pro Tyr Val Gly Arg Thr Lys Gly Glu Thr Ile Gly
 260 265 270
 35 Glu Met Ile Ala Ile Ser Asn Gln Phe Leu Arg Glu Leu Ser Ala Leu
 275 280 285
 Leu Lys Gln Gly Ala Gly Thr Lys Gly Ser Asn Lys Lys Lys Leu Leu
 290 295 300
 40 Ser Met Leu Ser Asp Tyr Trp Tyr Leu Ser Cys Gly Leu Leu Phe Pro
 305 310 315 320
 Lys Ala Glu Arg Tyr Asp Lys Ser Thr Trp Leu Thr Lys Thr Arg Asn
 325 330 335
 45 Ile Trp Ser Ala Pro Ser Pro Thr His Leu Met Ile Ser Met Ile Thr
 340 345 350
 Trp Pro Val Met Ser Asn Ser Pro Asn Asn Val Leu Asn Ile Glu Gly
 355 360 365
 50 Cys Pro Ser Leu Tyr Lys Phe Asn Pro Phe Arg Gly Gly Leu Asn Arg
 370 375 380
 Ile Val Glu Trp Ile Met Ala Pro Asp Glu Pro Lys Ala Leu Val Tyr
 385 390 395 400
 Ala Asp Asn Ile Tyr Ile Val His Ser Asn Thr Trp Tyr Ser Ile Asp
 405 410 415
 55 Leu Glu Lys Gly Glu Ala Asn Cys Thr Arg Gln His Met Gln Ala Ala
 420 425 430

Met Tyr Tyr Ile Leu Thr Arg Gly Trp Ser Asp Asn Gly Asp Pro Met
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 5 Phe Asn Gln Thr Trp Ala Thr Phe Ala Met Asn Ile Ala Pro Ala Leu
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 Val Val Asp Ser Ser Cys Leu Ile Met Asn Leu Gln Ile Lys Thr Tyr
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 10 Gly Gln Gly Ser Gly Asn Ala Ala Thr Phe Ile Asn Asn His Leu Leu
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 500 505 510
 15 Asp Ser Glu Glu Phe Lys Ser Ile Glu Asp Lys Leu Gly Ile Asn Phe
 515 520 525
 Lys Ile Glu Arg Ser Ile Asp Asp Ile Arg Gly Lys Leu Arg Gln Leu
 530 535 540
 20 Val Pro Leu Ala Gln Pro Gly Tyr Leu Ser Gly Gly Val Glu Pro Glu
 545 550 555 560
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 565 570 575
 25 Tyr Ser Lys Asp Leu Gly Ile Tyr Val Pro Val Leu Asp Lys Glu Arg
 580 585 590
 Leu Phe Cys Ser Ala Ala Tyr Pro Lys Gly Val Glu Asn Lys Ser Leu
 595 600 605
 30 Lys Ser Lys Val Gly Ile Glu Gln Ala Tyr Lys Val Val Arg Tyr Glu
 610 615 620
 Ala Leu Arg Leu Val Gly Gly Trp Asn Tyr Pro Leu Leu Asn Lys Ala
 625 630 635 640
 35 Cys Lys Asn Asn Ala Ser Ala Ala Arg Arg His Leu Glu Ala Lys Gly
 645 650 655
 Phe Pro Leu Asp Glu Phe Leu Ala Glu Trp Ser Glu Leu Ser Glu Phe
 660 665 670
 40 Gly Glu Ala Phe Glu Gly Phe Asn Ile Lys Leu Thr Val Thr Pro Glu
 675 680 685
 Ser Leu Ala Glu Leu Asn Arg Pro Val Pro Pro Lys Pro Pro Asn Val
 690 695 700
 45 Asn Arg Pro Val Asn Thr Gly Gly Leu Lys Ala Val Ser Asn Ala Leu
 705 710 715 720
 Lys Thr Gly Arg Tyr Arg Asn Glu Ala Gly Leu Ser Gly Leu Val Leu
 725 730 735
 50 Leu Ala Thr Ala Arg Ser Arg Leu Gln Asp Ala Val Lys Ala Lys Ala
 740 745 750
 Glu Ala Glu Lys Leu His Lys Ser Lys Pro Asp Asp Pro Asp Ala Asp
 755 760 765
 55 Trp Phe Glu Arg Ser Glu Thr Leu Ser Asp Leu Leu Glu Lys Ala Asp
 770 775 780

Ile Ala Ser Lys Val Ala His Ser Ala Leu Val Glu Thr Ser Asp Ala
 785 790 795 800
 5 Leu Glu Ala Val Gln Ser Thr Ser Val Tyr Thr Pro Lys Tyr Pro Glu
 805 810 815
 Val Lys Asn Pro Gln Thr Ala Ser Asn Pro Val Val Gly Leu His Leu
 820 825 830
 10 Pro Ala Lys Arg Ala Thr Gly Val Gln Ala Ala Leu Leu Gly Ala Gly
 835 840 845
 Thr Ser Arg Pro Met Gly Met Glu Ala Pro Thr Arg Ser Lys Asn Ala
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 15 Val Lys Met Ala Lys Arg Arg Gln Arg Gln Lys Glu Ser Arg Gln
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 <212> PRT
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 <223> /note="Consensus sequence of IBV VP5, whereby X stands for
 any amino acid"

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 20 25 30
 35 Asn Asn Arg Thr Gly Val His Ser Gly Arg His Pro Xaa Glu Ala His
 35 40 45
 Ser Gln Val Arg Asp Leu Asp Leu Gln Phe Asp Cys Gly Gly His Arg
 50 55 60
 40 Val Arg Ala Asn Cys Leu Phe Pro Trp Xaa Pro Trp Leu Asn Cys Gly
 65 70 75 80
 Cys Ser Leu His Thr Ala Glu Gln Trp Glu Leu Gln Val Arg Ser Asp
 85 90 95
 45 Ala Pro Asp Cys Pro Glu Pro Thr Gly Gln Leu Gln Leu Leu Gln Ala
 100 105 110
 Ser Glu Ser Glu Ser His Ser Glu Val Lys His Thr Xaa Trp Trp Arg
 115 120 125
 50 Leu Cys Thr Lys Xaa His His Lys Arg Arg Asp Leu Pro Arg Lys Pro
 130 135 140
 Glu
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 55 <210> 55
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<212> PRT
 <213> Infectious bursal disease virus

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 <223> /note="Sequence of IBDV D6948-VP5"

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 Asn Asn Arg Thr Gly Val His Ser Gly Arg His Pro Arg Glu Ala His
 35 40 45
 Ser Gln Val Arg Asp Leu Asp Leu Gln Phe Asp Cys Gly Gly His Arg
 50 55 60
 Val Arg Ala Asn Cys Leu Phe Pro Trp Phe Pro Trp Leu Asn Cys Gly
 65 70 75 80
 Cys Ser Leu His Thr Ala Glu Gln Trp Glu Leu Gln Val Arg Ser Asp
 85 90 95
 Ala Pro Asp Cys Pro Glu Pro Thr Gly Gln Leu Gln Leu Leu Gln Ala
 100 105 110
 Ser Glu Ser Glu Ser His Ser Glu Val Lys His Thr Pro Trp Trp Arg
 115 120 125
 Leu Cys Thr Lys Trp His His Lys Arg Arg Asp Leu Pro Arg Lys Pro
 130 135 140
 Glu
 145

<210> 56
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 <223> /note="Sequence of IBDV CEF94-VP5"

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 1 5 10 15
 Arg Ser Asn Pro Thr Asp Cys Ser Val His Thr Glu Pro Ser Asp Ala
 20 25 30
 Asn Asn Arg Thr Gly Val His Ser Gly Arg His Pro Gly Glu Ala His
 35 40 45
 Ser Gln Val Arg Asp Leu Asp Leu Gln Phe Asp Cys Gly Gly His Arg
 50 55 60
 Val Arg Ala Asn Cys Leu Phe Pro Trp Ile Pro Trp Leu Asn Cys Gly
 65 70 75 80

1. An infectious recombinant Infectious Bursal Disease Virus (rIBDV) essentially incapable of growing in a non-bursa-cell or cell derived thereof.
2. An infectious rIBDV having retained at least part of the very virulent characteristics of a very virulent Infectious Bursal Disease Virus (vvIBDV).
3. An rIBDV according to claim 1 having retained at least part of the very virulent characteristics of a very virulent Infectious Bursal Disease Virus (wIBDV).
4. An rIBDV according to anyone of claims 1 to 3 essentially incapable of growing in a CEF cell, a VERO cell or a QM5 cell.
5. An rIBDV according to anyone of claims 1 to 4 wherein the amino acid sequence of protein VP2 comprises no asparagine at amino acid position 279.
6. An rIBDV according to claim 5 wherein the amino acid sequence of protein VP2 comprises aspartic acid at amino acid position 279
7. An rIBDV according to anyone of claims 1 to 6 wherein the amino acid sequence of protein VP2 comprises no threonine at amino acid position 284.
8. An rIBDV according to claim 7 wherein the amino acid sequence of protein VP2 comprises alanine at amino acid position 284.
9. An rIBDV according to claim 8 wherein the amino acid sequence of protein VP2 at least comprises a stretch of amino acids from about position 279 to 289, preferably from about position 229 to 314, most preferably from about position 214 to 328 as found in a vvIBDV isolate such as shown in Table 8.
10. A method for obtaining an infectious recombinant Infectious Bursal Disease Virus (rIBDV) essentially incapable of growing on a non-bursa-cell derived cell comprising transfecting at least one first cell with a nucleic acid comprising a IBDV genome at least partly derived from IBDV, incubating said first cell in a culture medium, recovering rIBDV from said transfected first cell or said culture medium and propagating said recovered rIBDV in at least one second cell which is permissive for said rIBDV.
11. A method for obtaining an infectious recombinant Infectious Bursal Disease Virus (rIBDV) having retained at least part of the very virulent characteristics of a very virulent Infectious Bursal Disease Virus (vvIBDV) comprising transfecting at least one first cell with a nucleic acid comprising a IBDV genome at least partly derived from a vvIBDV, incubating said first cell in a culture medium, recovering rIBDV from said transfected first cell or said culture medium and propagating said recovered rIBDV in at least one second cell which is permissive for said vvIBDV.

12. A method according to claim 11 or 12 wherein said first cell is a non-bursa-cell derived cell.
13. A method according to anyone of claims 10 to 12 wherein said second cell is a Bursa-cell derived cell.
- 5 14. A method according to anyone of claims 10 to 13 wherein said first cell, such as a CEF cell, a VERO cell or a QM5 cell, is non-permissive for vvIBDV.
15. A method according to anyone of claims 10 to 14 wherein said first cell has additionally been provided with a
10 helpervirus or a viral protein derived thereof.
16. A method according to claim 15 wherein said viral protein comprises T7-polymerase.
17. A method according to anyone of claims 10 to 16 wherein said rIBDV has at least retained the incapacity to sub-
15 stantially be propagated on a vvIBDV non-permissive cell such as a VERO, a QM5 or CEF cell.
18. A method according to anyone of claims 10 to 17 wherein said permissive second cell is a primary bursa cell.
19. A method according to anyone of claims 10 to 18 wherein said rIBDV comprises at least a nucleic acid derived
20 from at least a part of genome segment A of vvIBDV.
20. A method according to claim 19 wherein said nucleic acid encodes at least a functional part of protein VP2.
21. A method according to anyone of claims 10 to 20 wherein said rIBDV comprises at least a nucleic acid derived
25 from a serotype II IBDV.
22. A method according to anyone of claims 10 to 21 wherein said rIBDV is lacking at least one immunodominant
epitope specific for a serotype I IBDV.
23. An infectious mosaic IBDV (mIBDV) comprising a rIBDV wherein at least one genome segment comprises nucleic
30 acid derived from at least two different Birna virus isolates.
24. A mIBDV according to claim 23 wherein at least one of said isolates is a wIBDV.
25. A mIBDV according to claim 23 or 24 characterised by its incapacity to substantially be propagated on a wIBDV
35 non-permissive cell such as a VERO, a QM5 or CEF cell.
26. A mIBDV according to anyone of claims 23 to 25 characterised by its capacity to substantially be propagated on
a vvIBDV permissive cell such as a primary bursa cell.
- 40 27. A mIBDV according to anyone of claims 23 to 26 wherein at least one of said isolates is a serotype II IBDV.
28. A mIBDV according to anyone of claims 23 to 27 lacking at least one immunodominant epitope specific for a
serotype I IBDV.
- 45 29. A vaccine comprising a rIBDV according to anyone of claims 1 to 9 or 23 to 28.

Figure 1: antibody titer in broilers (actively vaccinated with intermediate vaccine at 3 weeks)

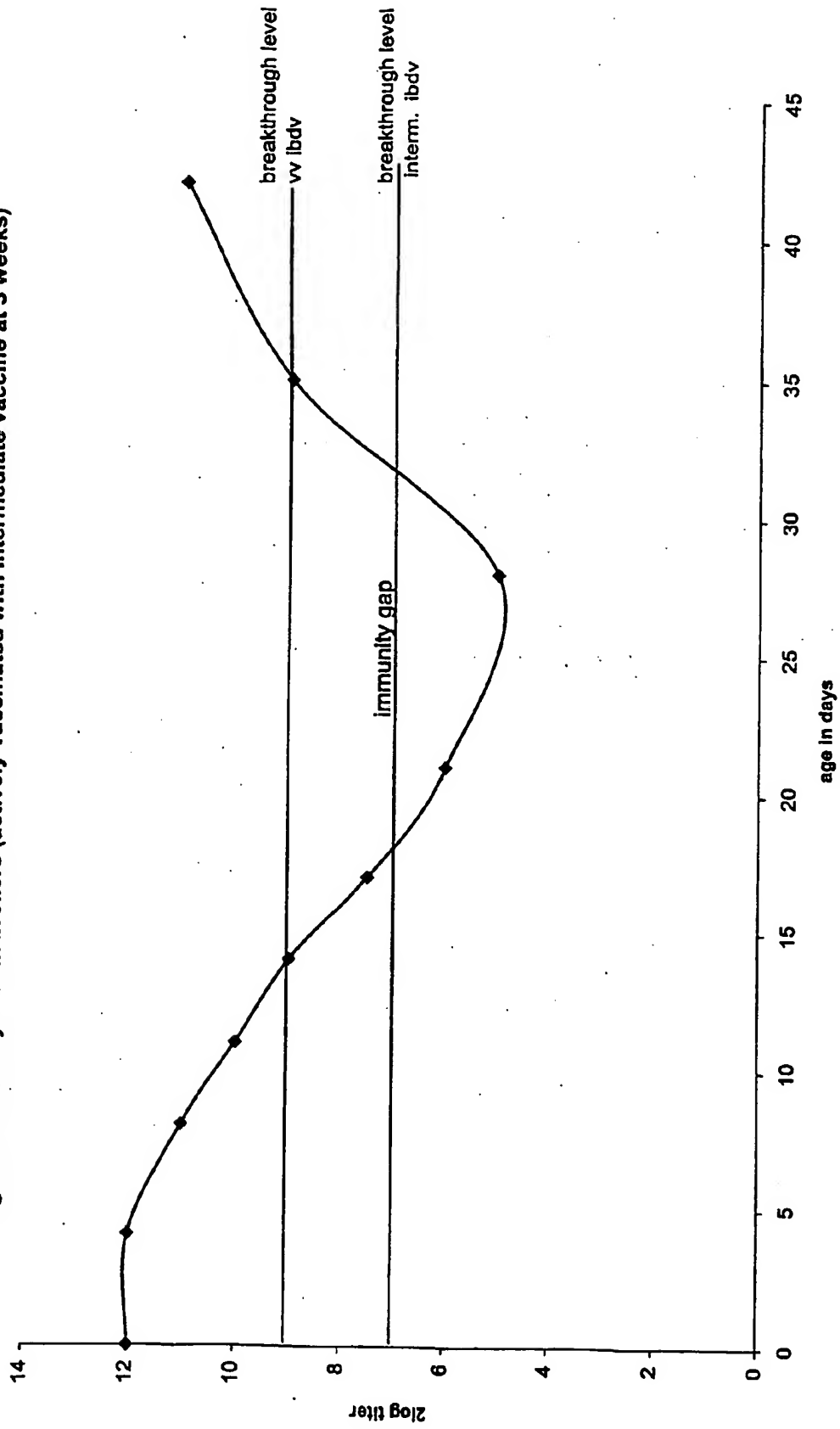


Fig. 2a Alignment of IBDV A-segment cDNA sequences

Consensus	GGATACGATC GGTCTGACCC CGGGGAGTC ACCCGGGAC AGGCYGMCAA GGYCTTGTTC CAGGATGGAA CTCTT	75
CEF94-AC.T... ..T.....	75
D6948-AT.A... ..C.....	75
TY89-A	-----	
Consensus	CCTTCTACAA YGCTATCATT GATGGTYAGT AGAGATCAGA CAAACGATCG CAGCGATGAC RAACCTGCAA GATCA	150
CEF94-AC.....C.....A.....	150
D6948-AT.....T.....G.....	150
TY89-A	-----	
Consensus	AACCCAAACAG ATTGTTCCGT TCATACGGAG CCTTCTGATG CCAACAACCG GACCGGGGTC CATTCCGGAC GACAC	225
CEF94-A	225
D6948-A	225
TY89-A	-----	
Consensus	CCTGAGAGAG CACACTCTCA GGTCAGAGAC CTCGACCTAC AATTGACTG TGGGGGACAC AGGGTCAGGG CTAAT	300
CEF94-A	...G.....	300
D6948-A	...A.....	300
TY89-A	-----	
Consensus	TGCTTTTTTC CCTGGTTTCC TTGGCTCAAT TGTGGGTGCT CACTACACAC TGCAGAGCAA TGGGAATAC AAGTT	375
CEF94-AA.....	375
D6948-AT.....	375
TY89-A	-----	
Consensus	CGATCAGATG CTCCTGACTG CCCAGAACCT ACCGGCCAGY TACAACCTACT GCAGGCTAGT GAGTCGGAGT CTCAC	450
CEF94-AT.....	450
D6948-AC.....	450
TY89-A	-----	
Consensus	AGTGAGGTCA AGCACACTYC CTGGTGGCGT TTATGCACTA AAYGGCACCA TAAAGCGCGT GACCTTCCAA GGAAG	525
CEF94-AT.....C.....	525
D6948-AC.....T.....	525
TY89-A	-----	
Consensus	CCTGAGTGAA CTGACAGATG TTAGCTACAA TGGGTGATG TCTGCAACAG CCAACATCAA CGACAAAATY GGGAA	600
CEF94-AT.....	600
D6948-AC.....	600
TY89-A	-----	
Consensus	CGTCCTAGTA GGGGAAGGGG TMACCGTCT CAGCTTACCC ACATCATATG ATCTTGGGTA TGTGAGRCTY GGTGA	675
CEF94-AC.....G..T.....	675
D6948-AA.....A..C.....	675
TY89-A	-----	
Consensus	CCCCATTCCT GCWATAGGGC TYGACCCAAA AATGGTAGCH ACATGTGACA GCACTGACAG GCCCAGAGTC TACAC	750
CEF94-AA.....T.....C.....	750
D6948-AT.....C.....A.....	750
TY89-A	-----	
Consensus	CATAACTGCA GCGATGATT ACCAATTCCT ATCACAGTAC CAASCAGGTG GGGTAACAAT CACACTGTTC TCAGC	825
CEF94-AC.....	825
D6948-AG.....	825
TY89-A	-----	
Consensus	YAAYATYGAT GCCATCACAA GCCTCAGCRT YGGGGGAGAR CTCGTGTTTC AAACAGCGT CCAHGGCCTT RTACT	900
CEF94-A	C..C..T... ..G..T.....G.....C.....G.....	900
D6948-A	T..T..C... ..A..C.....A.....A.....A.....	900
TY89-A	-----	
Consensus	GGGYGCTACC ATCTACCTYA TAGGCTTTGA TGGGACWCG GTAATCACCA GRGCTGTGGC CGCARACAAT GGGCT	975
CEF94-A	...C..C... ..C.....A.....G.....A.....	975
D6948-A	...T..T... ..T.....T.....A.....G.....	975
TY89-A	-----	

Fig. 2a Alignment of IBV A-segment cDNA sequences

Consensus	RACGRCGGGC	ACYGACAACC	TTWTGCCATT	CAATHTGTG	ATTCCAACHA	RCGAGATAAC	CCAGCCAATC	ACATC	1050
CEF94-A	G...A.....	.C.....	.T.....	.C.....	.A.....	A.....	.G.....	.T.....	1050
D6948-A	A...G.....	.T.....	.A.....	.A.....	.C.....	G.....	.G.....	.T.....	1050
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus	CATCAAACG	GAGATAGTGA	CCTCCAAAG	TGCTGGTCAG	GCRGGGGATC	AGATGTCRTG	GTCRGAAGW	GGGAG	1125
CEF94-AA.....	.G.....	.G.....	.A.....	1125
D6948-AG.....	.A.....	.A.....	.T.....	1125
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus	CCTAGCAGTG	ACGATCCAYG	GTGGCAACTA	TCCAGGGGCC	CTCCGTCCCG	TCACRCTAGT	RGCTACGAA	AGAGT	1200
CEF94-AT.....G.....	G.....	1200
D6948-AC.....A.....	A.....	1200
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus	GGCAACAGGA	TCYTCTGTTA	CGGTGCGYGG	GGTGAGCAAC	TTCGAGCTGA	TCCCAAATCC	TGAAGTACGA	AAGAA	1275
CEF94-AC.....	.T.....	1275
D6948-AT.....	.C.....	1275
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus	CCTGGTYACA	GAATACGGCC	GATTGACCC	AGGAGCCATG	AACTACACAA	AATTGATACT	GACTGAGAGG	GACCG	1350
CEF94-AT.....	1350
D6948-AC.....	1350
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus	TCTTGGCATC	AAGACCGTNT	GGCCAACAAG	GGAGTACACT	GACTTTGCGG	ARTACTTCAT	GGAGGTGGCC	GACCT	1425
CEF94-AC.....T.....	.A.....	1425
D6948-AA.....C.....	.G.....	1425
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus	CAACTCTCCC	CTGAAGATTG	CAGGAGCAIT	YGGCTTCAAA	GACATAATCC	GGGCCMTAAG	GAGGATAGCT	GTGCC	1500
CEF94-AC.....A.....	1500
D6948-AT.....C.....	1500
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus	GGTGGTCTCY	ACAYTGTTC	CACCYGCCGC	TCCCCTAGCC	CATGCAATTG	GGGAAGGTGT	AGACTACCTG	CTGGG	1575
CEF94-AC.....	.T.....	.T.....	1575
D6948-AT.....	.C.....	1575
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus	CGATGAGGCA	CAGGCTGCTT	CAGGAAGTGC	TCGAGCCCGG	TCAGGAAAG	CAAGAGCTGC	CTCAGGCCGC	ATAAG	1650
CEF94-A	1650
D6948-A	1650
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus	GCAGCTRACT	CTCGCCCGCG	ACAAGGGGTA	CGAGGTAGTC	GCGAATCTRT	TYCAGGTGCC	CCAGAATCCY	GTAGT	1725
CEF94-AG.....A.....	.C.....	.C.....	1725
D6948-AA.....G.....	.T.....	.T.....	1725
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus	CGACGGGATT	CTYGCTTCAC	CTGGGTACT	CCGCGGYGCA	CACAACCTCG	ACTGCGTGTT	RAGAGAGGGT	GCCAC	1800
CEF94-AT.....	.G.....	.T.....A.....	1800
D6948-AC.....	.A.....	.C.....G.....	1800
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus	GCTATTCCT	GTGGTYATYA	CGACAGTGA	AGAYGCCATG	ACACCCAAAG	CAYTGAACAG	CAAAATGTTT	GCTGT	1875
CEF94-AT..T.....C.....T.....	1875
D6948-AC..C.....T.....C.....	1875
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus	CATTGAAGGC	GTGCGAGAAG	RYCTCCAACC	TCCWTCTCAA	AGAGGATCCT	TCATACGAAC	TCTCTCYGGA	CAYAG	1950
CEF94-AC.....	.T.....T.....	.C.....	1950
D6948-AT.....	.A.....C.....	.T.....	1950
TY89-A	-----	-----	-----	-----	-----	-----	-----	-----	

Fig. 2a Alignment of IBDV A-segment cDNA sequences

Consensus	AGTCTATGGA TATGCTCCAG ATGGGGTACT TCCACTGGAG ACTGGGAGAG AYTACACCGT KGTCCCAATA GATGA	2025
CEP94-A	2025
D6948-A	2025
TY89-A	
Consensus	TGCTCTGGAC GACAGCATTA TGCTGTCCAA AGAYCCATA CCTCTATTG TGGGAACAG YGGAAAYCTA GCCAT	2100
CEP94-A	2100
D6948-A	2100
TY89-A	
Consensus	AGCTTACATG GATGTGTTTC GACCCAAAGT CCCHATCCAT GTGGCYATGA CGGGAGCCCT CAAYGCYTRT GGCGA	2175
CEP94-A	2175
D6948-A	2175
TY89-A	
Consensus	GATTGAGAAM GTRAGCTTTA GAAGCACCAA GCTGCCACT GCACACCGAC TTGGCCTYAA GTTGGCTGGT CCCGG	2250
CEP94-A	2250
D6948-A	2250
TY89-A	
Consensus	WGCATTYGA YTRAACACCG GGYCCAACTG GGCACGTTY ATCAAAGCTT TYCCTCACAA TCCCHGAGAC TGGGA	2325
CEP94-A	2325
D6948-A	2325
TY89-A	29
Consensus	CAGGYTHCCY TACCTCAACC TWCCHAYCT YCCACCHAMW GNGGACGYC AGTWCSAYCT KGCCHTGGCH GCHTC	2400
CEP94-A	2400
D6948-A	2400
TY89-A	104
Consensus	NGAGTTCAAA GAGACCCCMG AACTCGARRR YGCGTSMGW GCHATGGAGW CWGCGCHAA CGTSGACCCA YTRIT	2475
CEP94-A	2475
D6948-A	2475
TY89-A	179
Consensus	CCRHTCWGCD CTCHRBGTST TCATGTGGYT GGAAGARAAY GGGATTOTRA CYGAYATGGC YAACTTCGCM CTCAG	2550
CEP94-A	2550
D6948-A	2550
TY89-A	254
Consensus	CGACCCGAAC GCHCAIMGGA TGRHGAATT YCHGCAAA Y GNCCHCARG CHGCHAGCAA GTGCGARAGR GCCAA	2625
CEP94-A	2625
D6948-A	2625
TY89-A	329
Consensus	GTAYGGSACR GCGGCTACG GAGTGGAGGC YHGRGGCCCC ACDCAGARG ARGACAGAG GGARAAGAC ACACG	2700
CEP94-A	2700
D6948-A	2700
TY89-A	404
Consensus	GATCTCAAG AAGATGGARA CBATGGGCAT CTACTTYGCA ACACCRGAAT GGGTAGCACT CAAYGGGCAC CGRGG	2775
CEP94-A	2775
D6948-A	2775
TY89-A	479
Consensus	SCCAAGCCCC GGCCAGCTVA AGTACTGGCA RAACACANGA GAAATACCDG AHCCHACGA GGACTAYCYA GACTA	2850
CEP94-A	2850
D6948-A	2850
TY89-A	554
Consensus	YGTGCAYGCR GAQAAGAGCC GGTGGGCRTC AGAAGAACAR RTCTTAAGGG CAGCYACGTC GATCTACGGG GCTCC	2925
CEP94-A	2925
D6948-A	2925
TY89-A	629

Fig. 2a Alignment of IBDV A-segment cDNA sequences

Consensus	AGGACAGGCM GARCCACCCC AAGCTTTCAT AGACGAAGTY GCCARRGTCT ATGAAATCAA CCATGGRCGT GGYCC	3000
CEP94-AA ..G..... ..T..... ..T..... ..AA..... ..A..... ..C..	3000
D6948-AA ..G..... ..C..... ..C..... ..AA..... ..G..... ..C..	3000
TY89-AT ..A..... ..C..... ..C..... ..GG..... ..G..... ..T..	704
Consensus	MAACCARGAR CAGATGAARG AYCTGCTCTT GACTGCGATG GAGATGAAGC ATCGCAATCC CAGGCGGGCT CYACC	3075
CEP94-A	A.....A..AA. .T.....T.T...	3075
D6948-A	C.....A..AA. .T.....C.C...	3075
TY89-A	A.....G..GG. .C.....C.C...	779
Consensus	AAAGCCMAAG CCAAAACCCA ATGCTCCAMC ACAGAGACCC CCTGGWCGGC TGGGCCGCTG GATCAGRCB GTCTC	3150
CEP94-AC..A.T..... ..A.C	3150
D6948-AC..A.T..... ..G.T	3150
TY89-AA..T.A..... ..A.G	854
Consensus	TGAYGAGGAC YTKGAGTGAG GYWCCTGGGA GTCTCCCGAC ACCACCCGCG CAGGYGTGGA CACCAATTMR KDHHT	3225
CEP94-A	...T..... C.T..... .TA..... ..T..... ..CG GACT.	3225
D6948-A	...T..... C.T..... .CT..... ..C..... ..CG GCCA.	3225
TY89-A	...C..... T.G..... .CT..... ..T..... ..AA TCAC.	929
Consensus	ASWRMATYCS AAATTGGATC CGTTGCGGGG TCCCC	3260
CEP94-A	.CAAC..C.C	3260
D6948-A	.CAAC..C.C	3260
TY89-A	.GTGA..T.G	964

Fig. 2b Alignment of IBDV B-segment cDNA sequences

Consensus	GGATACGATG GGTCTGACCC TCTGGGAGTC ACGAATTAAC GTGGCTACTA GGGGYGATRM CCRCCGCTRG CTGCC	75
CEP94-BC...AA ..G....G.	75
D6948-BT...GC ..A....A.	75
Consensus	ACGTTAGTGG CTCCTCTTCT TGATGATTCT RCCACCATGA GTGACRTTTT CAAYAGTCCA CAGGCGCGAA GCANG	150
CEP94-B G..... ..A.... ..C..... ..C.	150
D6948-B A..... ..G.... ..T..... ..A.	150
Consensus	ATHTCAGCAG CGTTCGGCAT AAAGCCTACG GCTGGACARG AYTGGAAGA ACTCTGTATC CCTAARGTYT GGGTG	225
CEP94-B	..C..... ..T..... ..A.... ..C..... ..T..... ..A..T.	225
D6948-B	..A..... ..A..... ..G.... ..T..... ..C..... ..G..C.	225
Consensus	CCACCTGAGG ATCCSYTKGC CAGCCCTAGT CGWCTGGCHA AGTTCCTCAG RGARAACGGC TACAARRTTY TGCAG	300
CEP94-BGC.T.A....A.A..G.... ..AG..T.	300
D6948-BCT.G.T....C.G..A..... ..GA..C.	300
Consensus	CCACGGTCTC TRCCYGAGAA TGAGGAGTAT GAGACCGAYC AAATACTCCC WGACTAGCW TGGATGHRIC AGATA	375
CEP94-BG..C.... ..C..... ..A...T....AC.A.	375
D6948-BA..T.... ..T..... ..T...C....TA.G.	375
Consensus	GARGRGCTG TTTTAAACC MACYCTATCT CTCCTATTTG GAGAYCAGGA GTACTTCCW AARFACTACC CAACA	450
CEP94-B	..A..G.... ..C..T.... ..T.... ..T.... ..A.....A ..G..... ..	450
D6948-B	..G..A.... ..A..C.... ..C.... ..C.... ..T.....T ..A..... ..	450
Consensus	CATCGCCCKA GCAAGGARA GCGCAATGCG TACCCGCGMG AYATCGCAAT ACTCAAGCAG ATGATYTACY TGTTF	525
CEP94-B	..T....T.G.... ..A.... ..C.....C.T...C.	525
D6948-B	..C....G.A.... ..C.... ..T.....T.C...T.	525
Consensus	CTCCAGGTTT CHGAGGCCAM NGARRRCCTW AARGATGARG TNAACCTMYT RACCCAAAC ATWAGRGAYA ARGCC	600
CEP94-BA.....A C..GGG...A ..G....A. ..A....CT. G..... ..A..G..C. ..G... ..	600
D6948-BC.....C A..TAA...T ..A....G. ..C....AC. A..... ..T..A..T. ..A... ..	600
Consensus	TATGGRAGTG GGACCTACAT GGGACARGCM ACYNGACTTG TKGCTATGAA RGAGGTGCC ACTGGRAGAA ACCCA	675
CEP94-B	..T..A.... ..A....A ..TC.... ..G..C.... G....C.... ..A.... ..	675
D6948-B	..C..G.... ..G..C ..CA.... ..T..T.... A....T.... ..G.... ..	675
Consensus	AACAARGATC CTCFAAGCT TGGGTACACY TTTGAGAGCA THGSCAGCT ACTTGACATC ACWYACCGG TAGGC	750
CEP94-BG.... ..T..... ..C..G.... ..AC..... ..	750
D6948-BA.... ..C..... ..A..C.... ..TT..... ..	750
Consensus	CCACCCGGTG AGGATGACAA GCGCTGGGTR CCACTCACAA GRGTGCGGTC AHGGATGTTG GTWCTGACGG GNGAC	825
CEP94-BG..... ..A..... ..C..... ..A..... ..A... ..	825
D6948-BA..... ..G..... ..A..... ..T..... ..C... ..	825
Consensus	GTAGATGGSG AMFTTGAGGT TGARGAYTAC CTTCCCAAAA TCAACCTCAA GTCATCAAGT GGACTRCMT ATGTW	900
CEP94-BC. ..C..... ..A..T.... ..A.... ..A..A.A.	900
D6948-BG. ..A..... ..G..C.... ..A..... ..G..C.... ..T.... ..	900
Consensus	GGTCGCACCA AAGGAGARAC WATTGGSGAG ATGATAGCTA THTCRAACCA GTTCTTYNGA GAGCTATCAR CRCTG	975
CEP94-BG..A.... ..C.... ..T.... ..C..A.... ..CA.... ..A..A... ..	975
D6948-BA....T ..G.... ..C.... ..A..G.... ..TC.... ..G..G... ..	975
Consensus	YTGAAGCARG GTGCAGGAC AAARGGCTCR AACAAGAAGA AGCTRCTCAG CATGTTAAGT GACTATTGGT ACTTA	1050
CEP94-B	T.....A.G....AA..... ..T..... ..T..... ..	1050
D6948-B	C.....G.A....GG..... ..C..... ..C..... ..	1050
Consensus	TCATGYGGGC TTTTOTTTC MAAGGCTGAR AGGTACGACA AAGYACATG GCTCACCAG ACCCGKAACA TATGG	1125
CEP94-BC.... ..A.....AT..... ..G..... ..	1125
D6948-BT.... ..C.....GC..... ..T..... ..	1125
Consensus	TCAGCTCCAT CNCCAACACA CCTCATGATC TCWATGATHA CCTGGCCCGT GATGTCCAAY AGCCCAAYA ACCTG	1200
CEP94-BC..... ..T....C.C..... ..T.... ..	1200
D6948-BA..... ..A..... ..T..... ..C..... ..T..... ..	1200

Fig. 2b Alignment of IBV B-segment cDNA sequences

Consensus	TTGAACATTG ARGGGTGTCC RTCACTCTAC AARTTCAACC COTTYAGAGG WGGGYTAAAC AGGATCOTSG AGTGG	1275
CEF94-BA.....A.....A.....C.....A...T.G...C.....	1275
D6948-BG.....G.....G.....T.....T...C.A...G.....	1275
Consensus	ATAMTGGCYC COGANGAACG CAAGGCYYTW GTATATGCKG ACAACATATA CATTOTYCAC TCMACACAGT GGTAC	1350
CEF94-B	...T...C...A.....TC.T.....G.....C...A.....	1350
D6948-B	...A...T...T.....CT.A.....T.....T...C.....	1350
Consensus	TCAATGACC TAGAGAAGGG TGAGGCAAC TGCACKGVC AACACATGCA RGCCGCHATG TACTACATMC TYACC	1425
CEF94-BT.C.....A...A.....A...C...	1425
D6948-BG.T.....G.....C.....C...T...	1425
Consensus	AGAGRTGGT CMGAYAACGG YGACCCATG TTCAATCARA CATGGGCCAC CTTTGCATG AACATTGCCG CMGCT	1500
CEF94-B	...G....A..C....C....A...A.....C.....T...	1500
D6948-B	...A....C..T....T....C...G.....G.....A...	1500
Consensus	CTAGTGTGG ACTCATCTG YCTGATGATG AACCTKCARA TYAGACHTA TGGTCAAGGC AGYGGGAATG CAGCC	1575
CEF94-B	...G....G....C....A...G..A..T...C...C.....	1575
D6948-B	...T....A...T....T...T..G..C....A...T.....	1575
Consensus	ACSTTCATCA ACAACCAYCT YYTKAGCACG CTWGTGCTWG ACCAGTGGAA CYTGATGARR CARCCYAGNC CAGAC	1650
CEF94-B	..G.....C...CT.G....G..A...T.....C.....GA..G..C..A...	1650
D6948-B	..C.....T...TC.T....C..T...A.....T.....AG..A..T..T....	1650
Consensus	AGCGARGAGT TCAATCAAT TGAGACAAAG CTROGYATCA ACTTYAAGAT TGAGAGOTCC APTGATGAYA TYAGG	1725
CEF94-B	...G....A....G....A...T...T.....T.....T...C...	1725
D6948-B	...A....G....A....G..C....C.....C.....C...T...	1725
Consensus	GGCAAGCTSA GACAGCTTGT CCYCCCTGCA CAACCAGGCT ACCTGAGTGG RGGGGTYGAA CCAGARCAAY CCAGC	1800
CEF94-BG.....T.....G.....T...A...T....	1800
D6948-BC.....C.....A.....C.....G...C....	1800
Consensus	CCAACTGTWG AGCTKGACCT ACTHGGRTGG TCMGCHACWT ACAGCAAAGA TCTYGGGATC TATGTGCCGG TGCTT	1875
CEF94-BT...T....A..G...A..T..A.....C.....	1875
D6948-BA...O....C...A...T..A..T.....T.....	1875
Consensus	GACAAGGAAC GCYTATTITG YTCGTCTGCG TATCCCAARG GRGTAGAGAA YAARAGYCTC AARTCCAARG TYGGG	1950
CEF94-BC.....T.....G..A.....C..G..T...G...A..C...	1950
D6948-BT.....C.....A...G.....T..A..C...A...G..T...	1950
Consensus	ATCGAGCARG CATACAARGT WGTCAAGTAY GAGGCGTTGA GGTGGTAGG TGGTTGGAAC TACCACTCC TGAAC	2025
CEF94-BG.....G..A.....T.....T.....T.....	2025
D6948-BA.....A...T.....C.....T.....T.....	2025
Consensus	AAAGCTTGCA AGAAYAAAGC ARGYGCHGCT CGGCGGCATC TGGAGGCCAA GGGGTTCR CCGAYGAGT TCTTN	2100
CEF94-BC.....T..C...G.C..C.....A.....C.....A	2100
D6948-BT.....C..T...A..T..A.....G.....T.....C	2100
Consensus	GCCGATGGT CMGAGYTOTC MGAGTTCGGW GARGCYTTCG AAGGCTTCAA YATCAAGCTG ACMGTAAACAY CKGAG	2175
CEF94-BT...C...A.....T..G..C.....T.....C.....T..T...	2175
D6948-BA..T...C.....A..A..T...C.....A.....C..G...	2175
Consensus	AGCCTHGCCG AACTKAACAR RCCAGTACCC CCCAARCCYC CAAATGTCAA CAGACCAGTC AACACYGGKG GRTTN	2250
CEF94-B	...A....G....A.G.....G..C.....T...G..A..C	2250
D6948-B	...C....T....G.A.....A..T.....C...T...G..A	2250
Consensus	AAGGCAGTCA GCAAYGCCCT CAAGACCGGY CGGTAYAGRA AYGAAGCCGG ACTRAGTGGY CTGCTCTYC TAGCC	2325
CEF94-BC.....T.....C..G..C.....G....T.....T.....	2325
D6948-BT.....C.....T..A..T.....A....C.....C.....	2325
Consensus	ACNGCHMGHA GCCGWTCTCA RGAYGCASTY AAGGCCAAGG CAGARGCCGA GAAACTCCAC AAGTCYAAGC CMGAY	2400
CEF94-B	..A..AA.A...T..G..A..T...T.....A.....C....A..C	2400
D6948-B	..C..CC.C...A..A..G..C....C.....G.....T....C..T	2400

Fig. 2b Alignment of IBDV B-segment cDNA sequences

Consensus	GACCCCGATG CAGACTGGTT YGAAMERTCA GAAACYCTGT CAGACCTKCT GGAGAAAGCC GACATYGCCA GCAAG	2475
CEF94-B C...A..A... ..T... ..T... ..C....	2475
D6948-B T...C..G... ..C.... ..G... ..T....	2475
Consensus	GTGGCYCACT CAGCACTCGT GGAACAAGC GAGGCYCTTG AAGCRGTYCA GTCRACYTCH GTGTACACYC CHAAG	2550
CEF94-BC.... ..C.... ..A..T... ..G..T..C ..T... .C...	2550
D6948-BT.... ..T.... ..G..C... ..A..C..AC. .A...	2550
Consensus	TACCCAGARG TYAAGAAGCC ACAGACCGCC TCCAACCCCG TTGTTGGGCT CCACCTGCCC GCCAAGAGRG CCACC	2625
CEF94-BA. .C..... ..T..... ..A.....	2625
D6948-BG. .T..... ..G.....	2625
Consensus	GGGTCCAGG CHGCTCTTCT CGGAGCAGGR ACGAGCAGAC CAATGGGGAT GGAGGCYCCA ACACGGTCCA AGAAC	2700
CEF94-BC..... ..A..... ..C....	2700
D6948-BA..... ..G..... ..T....	2700
Consensus	GCCGTGAAAA TGGCCAAAGG GCGGCAAGC CAAAARGAGA GCGGCCAAYA GCCATGATGG GAACCACTCA AGAAG	2775
CEF94-BC.G.... ..C....	2775
D6948-BA.A.... ..T....	2775
Consensus	AGGACACTAA YCCCAGACCC CGTATCCCGG GCCTTCGCCT GCGGGGGGCC CC	2827
CEF94-B T..... ..	2827
D6948-B C..... ..	2827

Fig. 3a IBDV polyprotein alignment

Consensus	MTNLQDQTQQ IVPFIRSLIM PTTGPASIPD DTLEKHTLRS ETSTYNLTVG DTGSGLVVFP PGFPGSIVQA HYTLQ	75
CEP94-PP	75
D6948-PP	75
TY89-PP	
Consensus	SNGNYKFDQM LLTAQNLPS YNYCRLVRSR LTVRSSTLEPG GUYALNOTIN AVTPQGSLSB LTDVSYNGLM SATAN	150
CEP94-PP	150
D6948-PP	150
TY89-PP	
Consensus	INDKIGNVLV GEGVTLSLP TSYDLGYVRL GDPIPAIGLD PKMVATCDSS DRPRVTITTA ADDYQFSSQY Q.GGV	225
CEP94-PP	225
D6948-PP	225
TY89-PP	
Consensus	TITLPSANID AITSLS.GGE LVFQTSV.GL .LGATIIYLIG FDGTAVITRA VAA.NGLT.G TDNL.PFN.V IPT.E	300
CEP94-PPV.....H.....V.....N.....T.....L.....L.....N.....	300
D6948-PPI.....Q.....I.....D.....A.....M.....I.....S.....	300
TY89-PP	
Consensus	ITQPITSIKL EIVTSKSGGQ AGDQMSWA. GSLAVTINGG NYPGALRPVT LVAYERVATG SVVTVAGVSN FELIP	375
CEP94-PPR.....	375
D6948-PPS.....	375
TY89-PP	
Consensus	NPFLAQNLTV EYGRFDPGAM NYTKLILSER DRIGIKTVNP TREYTDPREY PKEVADLNSP LKAGAPGPK DIIRA	450
CEP94-PP	450
D6948-PP	450
TY89-PP	
Consensus	.RRIAVPVVS TLPFPAAPLA HAIGGVVDYL LGDEAQAASG TARAASGAR AASGRIRQLT LAADKGYEVV ANLFQ	525
CEP94-PP	I.....	525
D6948-PP	L.....	525
TY89-PP	
Consensus	VPQNPPVDGI LASPG.LRGA HNLDCLVREG ATLFPVVITT VEDAMTFKAL NSKMPAVIEG VREDLQPPSQ RGSFI	600
CEP94-PPV.....	600
D6948-PPI.....	600
TY89-PP	
Consensus	NTLSGHRVYG YAPDGVLPLE TGRDYTVVPI DDVMDSIML SKDPIPIVG NSGNLAIAYM DVFRPKVPIH VAMTG	675
CEP94-PP	675
D6948-PP	675
TY89-PP	
Consensus	ALMA.GEIE. VSFRTKLAT ARLGLKLAG PGAPDVNTG. NWATFIKRPF HNPRDWRLLP YINLPYLPFN AGRQY	750
CEP94-PPC....K.....P.....	750
D6948-PPY....N.....S.....	750
TY89-PPT....P.....	28
Consensus	HLMAASEPK ETPLESAYR AMEAAANVDP LFQSALSVM WLEENGIVTD MANFALSDPN AHRMNFAN APQAG	825
CEP94-PP	825
D6948-PP	D.....	825
TY89-PP	..L.....D....D.....R...Q....K.....	103
Consensus	SKSQRAKYGT AGYGYEARGP TPEEAQREKD TRISKUMETH GIYPATFEWV ALNGHRGPSP GQLKYMONTA EIPDP	900
CEP94-PP	900
D6948-PP	900
TY89-PPE.....	178
Consensus	NEDYLDYVHA EKSRLASEQ ILRAATSIYG APGQAEPPQA FIDEVAKVYE INHGRGPNQS QMKDULLTAM EMKHA	975
CEP94-PP	975
D6948-PP	975
TY89-PPF.....V.....R.....	253
Consensus	NPRRAFFPKK PKPNAPTQRF PGRLGRMIRT VSDLE	1012
CEP94-PPL.....	1012
D6948-PPA.....	1012
TY89-PPS.....	290

Fig. 3b IBDV VP1 alignment

Consensus	MSD.FNSPQA RS.ISAAPGI KPTAGQDVEE LLIPKVVWPP EDPLASPSRL AKFLRENGYK .LQFRSLPEN KEYET	75
CEF94-VP1	...I.....T.....V.....	75
D6948-VP1	...V.....K.....I.....	75
Consensus	DQILPDLAMH RQIEGAVLKP TSLPIGDQE YPFKYYPTHR PSKEKPNAYP PDIALLRQMI YLFLQVPEA. .LKD	150
CEF94-VP1N EG...	150
D6948-VP1T DN...	150
Consensus	EVTLLTQHIR DKAYGSGTYM GQATRLVAMK EVATGRNPNK DPLKLGYPPE SIAQLLDITL FVGPPGEDDK FNVPL	225
CEF94-VP1	225
D6948-VP1	225
Consensus	TRVPSRMLVL TGDVDG.FEV EDYLPKINLK SSSGLPYVGR TKGETIGEMI AISNQFLREL S.LLKQGAGT KGSNK	300
CEF94-VP1D.....T.....	300
D6948-VP1E.....A.....	300
Consensus	KKLLSHLSDY WYLSGGLLFP KAERYDKSTW LTKTRNIWSA PSPTHLMISH ITWPNVNSNP NNVLNIEGCP SLYKP	375
CEF94-VP1	375
D6948-VP1	375
Consensus	NPFRRGLNRI VEWI.AP.EP KALVYADNIY IVHSNTWYSI DLEKGEANCT RQHNQAANYI ILTRGWSING DPMFN	450
CEF94-VP1L..E.....	450
D6948-VP1M..D.....	450
Consensus	QTWATFAMNI APALVVDSSC LIMNLQIKTY GQSGNAATP INNHLSTLV LDQNLN.QP .PDSEEFKSI EDKLG	525
CEF94-VP1R.. R.....	525
D6948-VP1X.. S.....	525
Consensus	INPKIERSID DIRGKLRQLV .LAQPGYLSG GVEPEQ.SPT VELDLIGWSA TYSKDLQIYV FVLDERLPC SAAYP	600
CEF94-VP1L.....S.....	600
D6948-VP1P.....P.....	600
Consensus	KGVNKSLSKS KVGIEQAYKV VRYEALRLVG GWNYPLINKA CKNNA.AARR HLEAKGFFLD EFLAEWSELS EFGEA	675
CEF94-VP1G.....	675
D6948-VP1S.....	675
Consensus	FEGFNKLTV T.ESLAEIN. PVPPKPPNVN RPNVTGGLKA VSNALKTGRY RNEAGLSGLV LLATARSRLQ DAVKA	750
CEF94-VP1S.....K.....	750
D6948-VP1P.....R.....	750
Consensus	KAEAEKLKKS KPDDPDADWF ERSETLSDLL EKADIASKVA HSAIVETSDA LEAVQSTSVY TEKYPEVKNP QTASN	825
CEF94-VP1	825
D6948-VP1	825
Consensus	FVVGLHLPK RATGVQAALL GAGTSRPMGN EAPTRSKNAV KMARRQRQK ESRQ..	881
CEF94-VP1QP	881
D6948-VP1--	879

Fig. 3c IBDV VP5 alignment

Consensus	MVSRDQTNDR SDD.PARSNP TDCSVHTEPS DANNRTGVHS GRHP.EAHSQ	50
D6948-VP5E.....R.....	50
CEF94-VP5K.....G.....	50
Consensus	VRDLDLQFDC GGHRVRANCL FPW.PWLNCG CSLHTAEQWE LQVRSDAPDC	100
D6948-VP5F.....	100
CEF94-VP5I.....	100
Consensus	PEPTGQLQLL QASESESHSE VKHT.WWRLC TK.HHKRRDL PRKPE	145
D6948-VP5P.....W.....	145
CEF94-VP5S.....R.....	145

Fig. 4 Schematic representation of the used plasmids

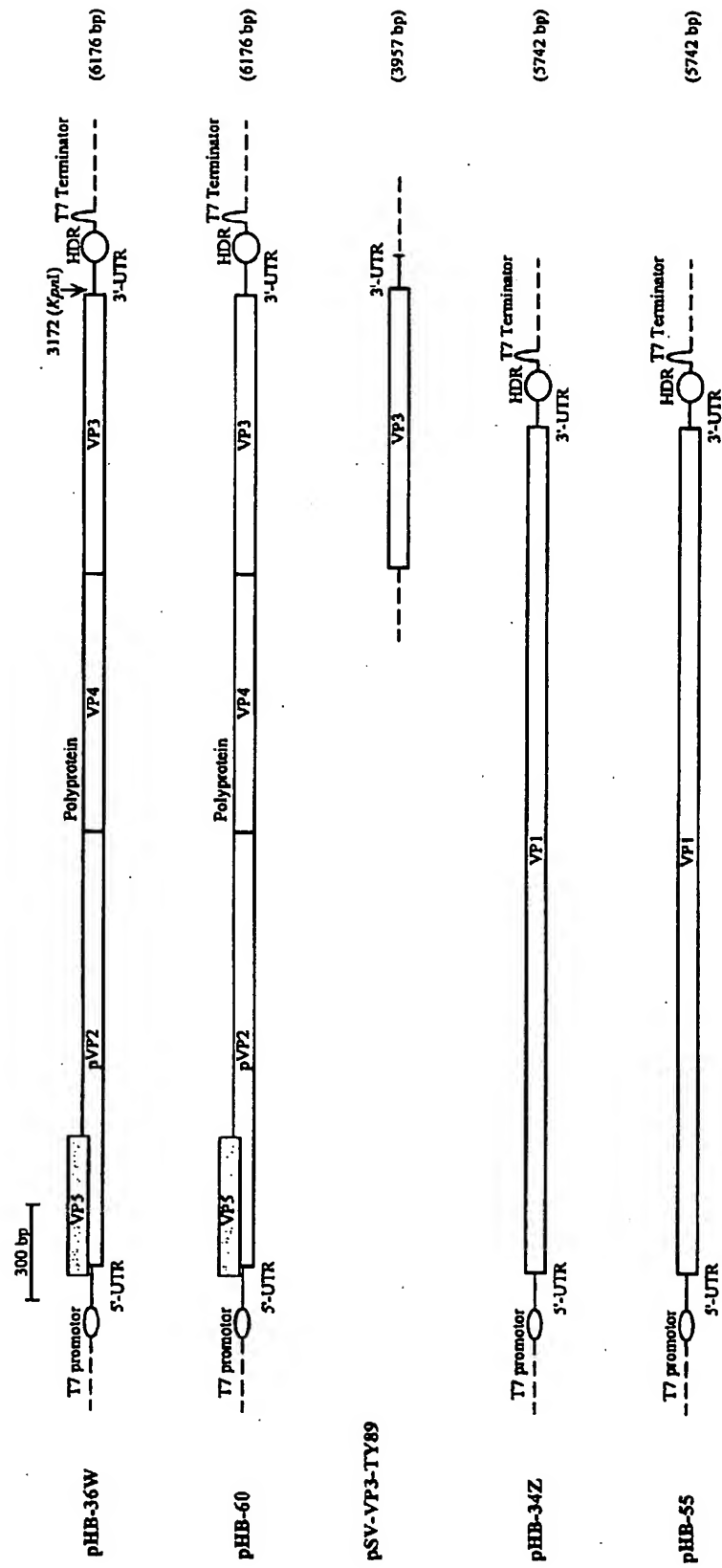


Fig. 5a Schematic representation of the construction of PCR fragment PCR-VP2d

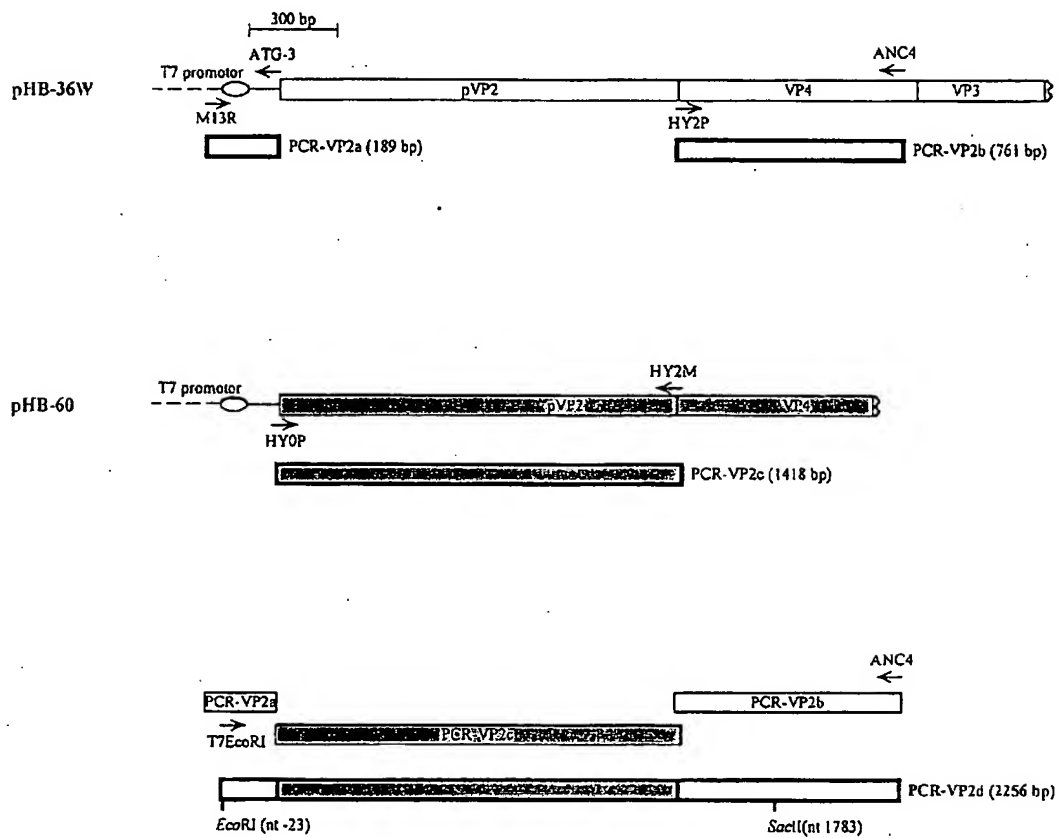


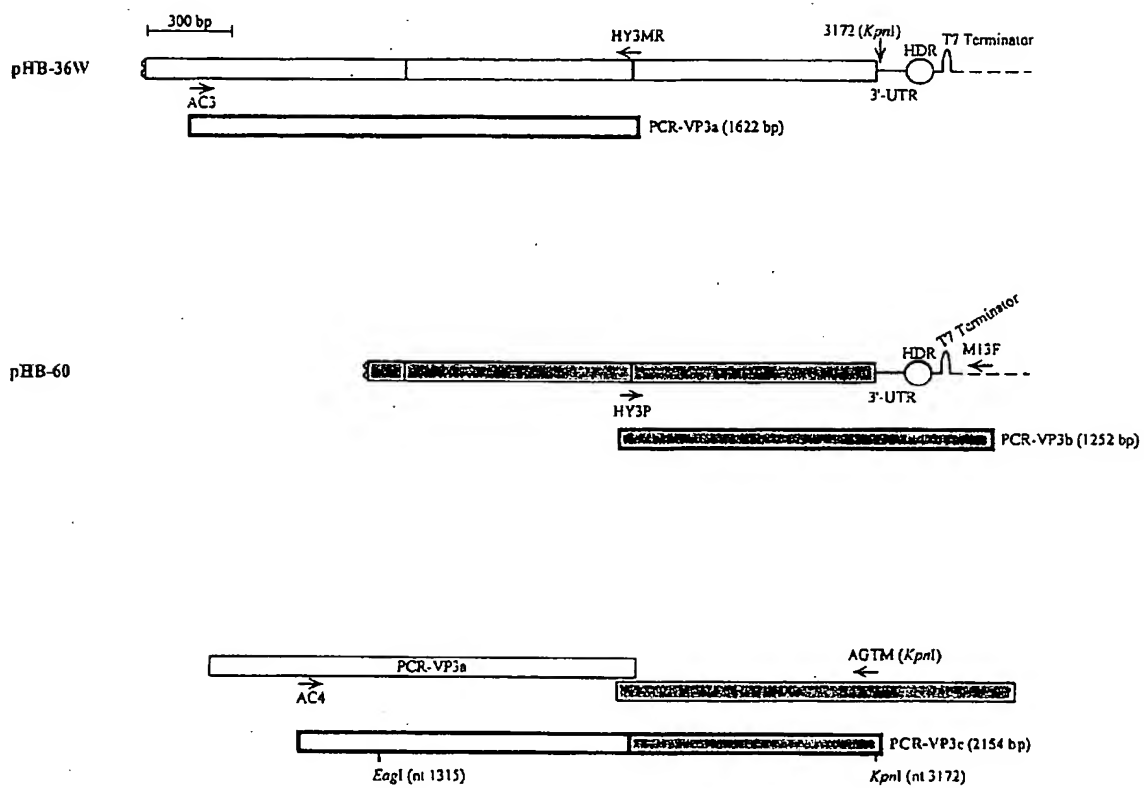
Fig. 5b Schematic representation of the construction of PCR fragment PCR-VP3c

Fig. 5c Schematic representation of the construction of PCR fragment PCR-VP4d

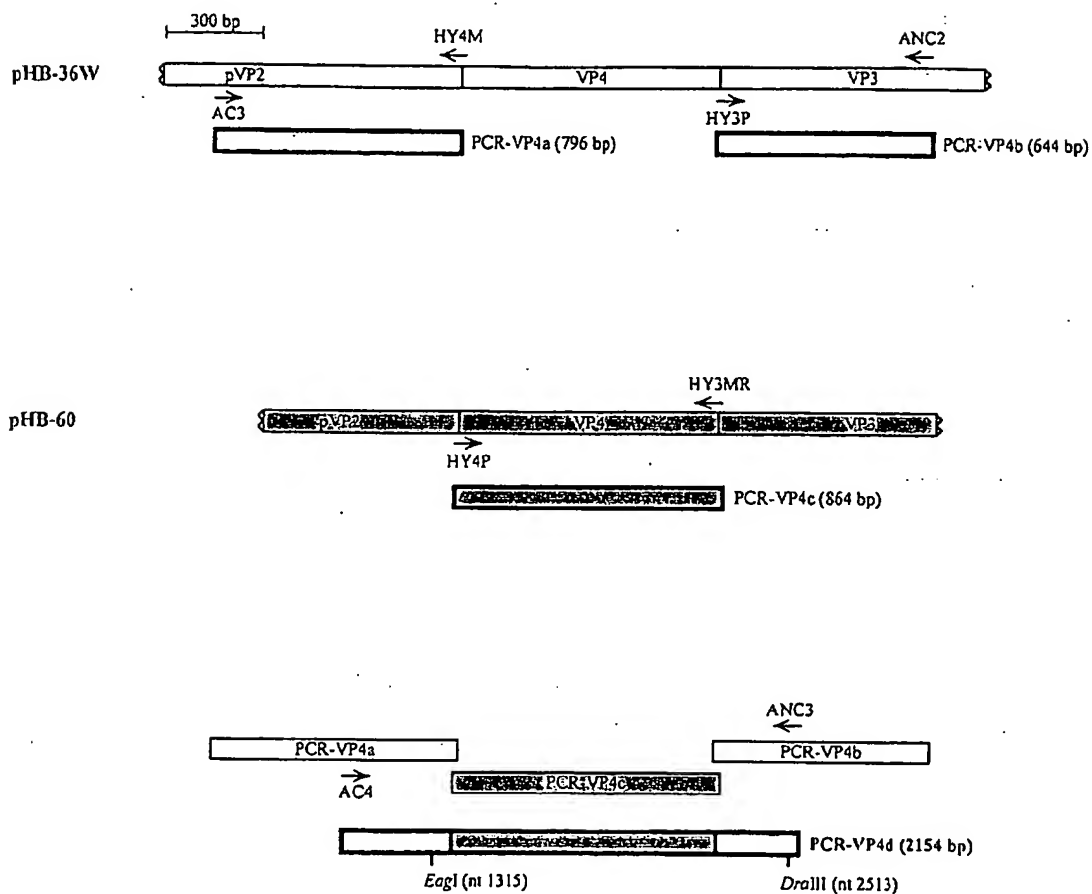


Fig. 5d Schematic representation of the construction of plasmid pHB36-s2VP3

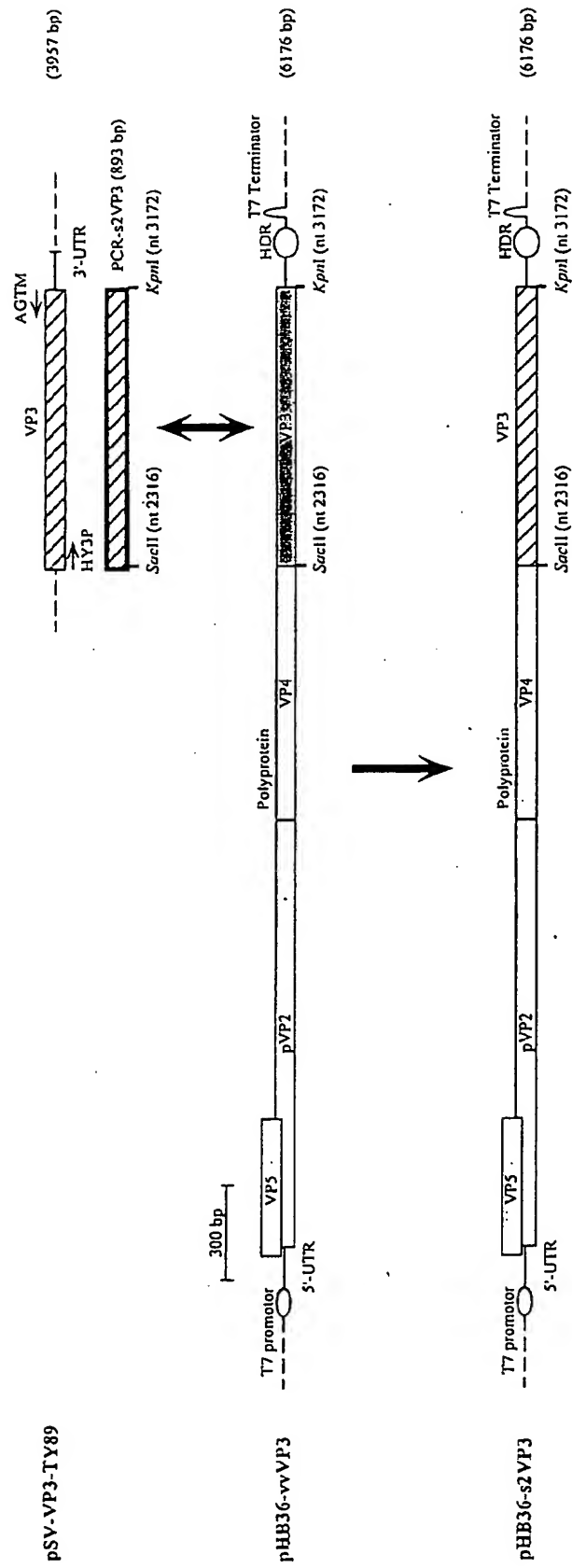


Fig. 5e

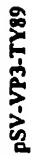


Fig. 5f Schematic representation of the construction of plasmid pHB36-s2VP3N

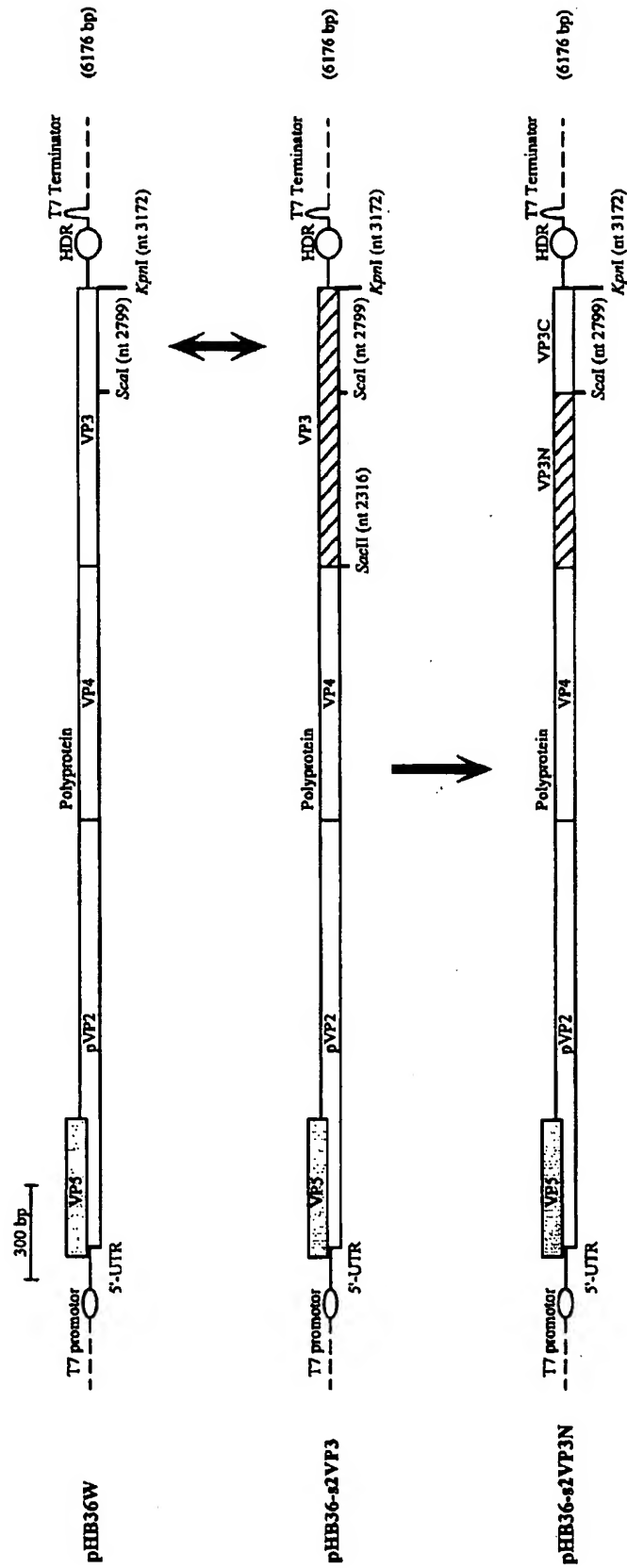
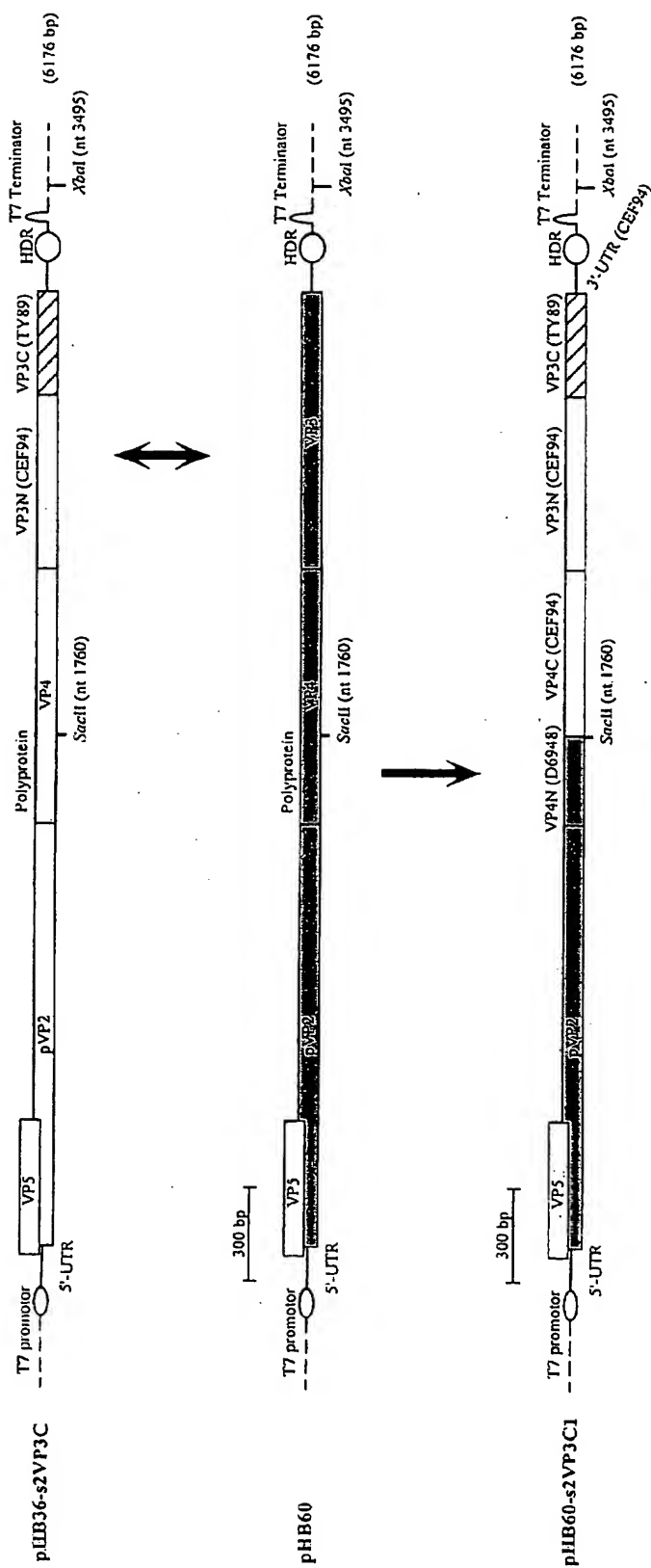


Fig. 5g Schematic representation of the construction of plasmid pHB60-s2VP3C1





European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 99 20 2316

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	LIM, B-L. ET AL.: "Adaptation of very virulent infectious bursal disease virus to chicken embryonic fibroblasts by site-directed mutagenesis of residues 279 and 284 of viral coat protein VP2." JOURNAL OF VIROLOGY, vol. 73, no. 4, April 1999 (1999-04), pages 2854-62, XP002126883	1-9, 23-29	C12N15/40 C12N7/00 C07K14/08 A61K39/12
Y	* the whole document *	10-15, 17-22	
Y	US 4 824 668 A (MELCHIOR JR FRED W ET AL) 25 April 1989 (1989-04-25) * column 1, paragraph 3 *	10-15, 17-22	
X	WO 98 09646 A (MUNDT EGBERT ;VAKHARIA VIKRAM N (US); UNIV MARYLAND BIOTECHNOLOGY) 12 March 1998 (1998-03-12)	1-9, 23-29	
A	* see whole document, particularly claims and page 15, line 18 - page 17 *	16	
X	WO 98 06827 A (UNIV MINNESOTA) 19 February 1998 (1998-02-19) * the whole document *	10-22	C12N A61K C07K
A	WO 95 26196 A (UNIV MARYLAND) 5 October 1995 (1995-10-05) * the whole document *		
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 6 January 2000	Examiner Smalt, R
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

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**ANNEX TO THE EUROPEAN SEARCH REPORT
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EP 99 20 2316

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

06-01-2000

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